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Contents

A Critical Comparison of the T-1824 Dye and Iodinated Albumin Methods for Plasma Volume Measurement. ALVIN L. SCHULTZ, JAMES F. HAMMARSTEN, BEN I. HELLER, AND RICHARD V. EBERT 107

Electrolyte Equilibria in Erythrocytes During Diabetic Acidosis. GEORGE NICHOLS, JR., AND NANCY NICHOLS 113

Observations Concerning the Cholate:Cholesterol Relationship in Clinical and Experimental Nephrosis. RAY H. ROSENMAN, MEYER FRIEDMAN, AND SANFORD O. BYERS 121

The Effects of Intravenous Priscoline on Cerebral Circulation and Metabolism. P. SCHEINBERG, I. BLACKBURN, AND M. RICH 125

Studies of Pulmonary Hypertension. I. Pulmonary Circulatory Dynamics in Patients with Pulmonary Emphysema at Rest. PAUL N. G. YU, FRANK W. LOVEJOY, JR., HOWARD A. JOOS, ROBERT E. NYE, JR., AND WILLIAM S. MCCANN 130

Simultaneous Distribution of T-1824 and I^{131}-Labelled Human Serum Albumin in Man. NORBERT FREINKEL, GEORGE E. SCHREINER, AND JOHN W. ATHENS 138

The Effect of a Thyrotrophic Hormone Preparation on the Metabolism of Radioiodine in Euthyroid, Hyperthyroid and Acromegalic Individuals. DAVID V. BECKER, J. E. RALL, WENDELL PEACOCK, AND RULON W. RAWSON 149

A Method for Determining Cardiac Output by the Direct Fick Principle without Gas Analysis. JACOB GROSSMAN, RAYMOND E. WESTON, AND LOUIS LEITER 161

Hexamethonium—Its Effect on Glomerular Filtration Rate, Maximal Tubular Function, and Renal Excretion of Electrolytes. JOHN H. MOYER AND LEWIS C. MILLS 172

Dietary Treatment of Hypertension. III. The Effect of Protein on Appetite and Weight. VINCENT P. DOLE, LEWIS K. DAHL, IRVING L. SCHWARTZ, GEORGE C. COTZIAS, JØRN H. THAYSEN, AND CECILIA HARRIS 185

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PATIENTS WHO TAKE THIOMERIN AT HOME BENEFIT BY SMOOTH MAINTENANCE OF MERCURIAL-DIURETIC EFFECTS

According to many physicians, the objectives of management with a mercurial diuretic are twofold:

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2 to guard against critical shortages of electrolytes that may occur if the sudden excretion of large volumes of urine is repeatedly induced. It is now recognized that excessive losses of electrolytes are responsible for certain "overtreatment complications" that sometimes interfere with the successful management of patients taking digitalis and diuretics.[1] (Sodium elimination may actually be *impeded* if total electrolytes become inadequate for maintaining a good filtration rate in the kidneys; inadvertent *potassium* depletion often results in abnormal responses to digitalis; generalized symptoms such as weakness, restlessness and cramps may also result if the concentrations of the various electrolytes have been thrown out of proportion.)

Achieving these objectives generally calls for the administration of a suitable diuretic in small doses at frequent intervals, just as maintenance doses of digitalis are used. With the mercurial, as with digitalis, the aim is at keeping the patient continuously free from any symptoms of congestive heart failure, instead of allowing him to "relapse" between doses. For this purpose, the thionated mercurial diuretic, THIOMERIN, is widely preferred because it is so dependable in action and so well tolerated by most patients *when given subcutaneously*, as well as when given intramuscularly and intravenously.

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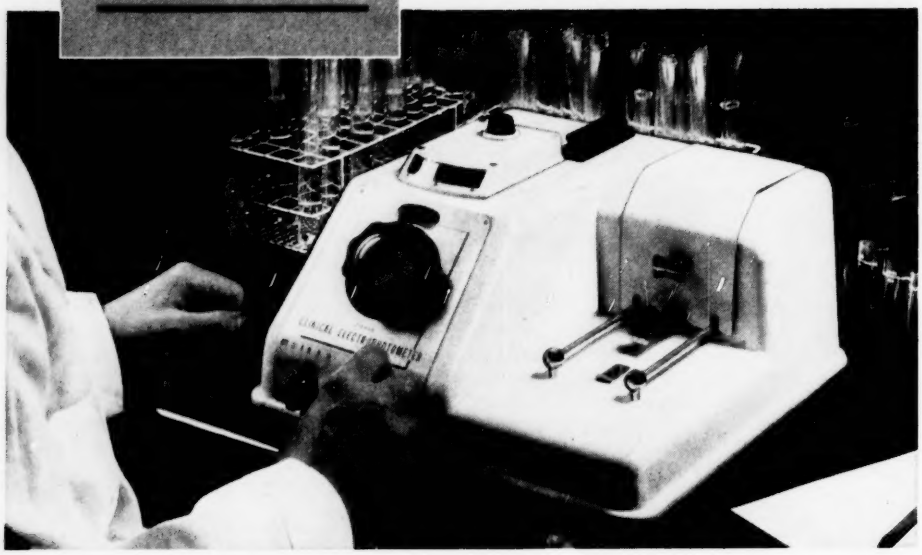

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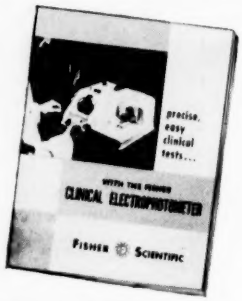

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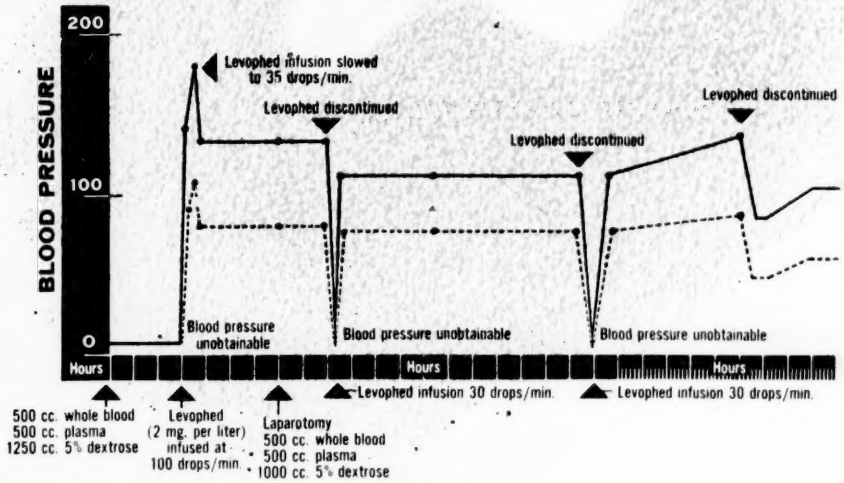

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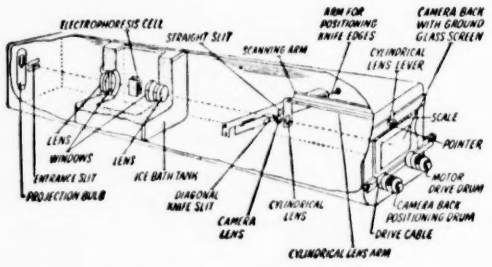

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A CRITICAL COMPARISON OF THE T-1824 DYE AND IODINATED ALBUMIN METHODS FOR PLASMA VOLUME MEASUREMENT [1]

By ALVIN L. SCHULTZ, JAMES F. HAMMARSTEN, BEN I. HELLER, AND RICHARD V. EBERT

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(Submitted for publication August 1, 1952; accepted November 3, 1952)

The T-1824 dye method for plasma volume measurement has been subjected to much criticism. The validity of its use as a tagged protein dilution method has been questioned. One of the major criticisms has been that part of the injected dye is rapidly removed from the plasma before binding with protein occurs. This objection does not apply to the use of human serum albumin tagged with radioactive iodine before injection. Comparison of the two methods has given conflicting results (1–3). The present study was undertaken to clarify the relationship between the dye and the iodinated albumin methods for the measurement of the plasma volume.

METHODS

A modification of the T-1824 dye method described by Gibson and Evelyn (4) was used in the present study. Two cc. of an aqueous solution containing 10 mg. of T-1824 dye [2] was measured in a calibrated syringe and injected intravenously. Blood samples were then drawn at 10-minute intervals for 1 hour by interrupting a continuous, slow, intravenous infusion of isotonic saline. The blood samples were collected in 4 cc. hematocrit tubes containing 1.25 mg. of heparin. The plasma was separated by centrifugation for 30 minutes at 3500 r.p.m. The plasma containing dye was compared with a blank plasma sample drawn just prior to the dye injection. An Evelyn colorimeter with a 620 millimicra filter was used. The optical densities were plotted against time on semilogarithmic paper and extrapolated to zero time (C_0). The optical density of a 1:1000 dilution of T-1824 dye in plasma was determined (K). The K factor of the dye was determined individually in the plasma of each of 21 normal subjects. The mean value for the individual K factors was 0.351 (S.D. 0.009). This mean K was then used in the calculation of the plasma volume in 13 patients in whom an individual K had not been determined. An individual K factor was determined in 15 patients and utilized in the calculation of the plasma volume. The K factor determined with plasma containing 1 gm. per cent of polyvinylpyrrolidone (PVP) did not differ significantly from that using plasma alone. A microadapter (5) for the Evelyn macrocolorimeter was used in all colorimetric measurements so that a 1 cc. plasma sample could be utilized. The calculation of the plasma volume was based on the following equation (6):

$$\text{Plasma volume (cc.)} = \frac{K \times 1000 \times \text{cc. of dye injected}}{C_0}$$

The I^{131} tagged human serum albumin used in this study was supplied by Abbott Laboratories, Chicago, Ill. The solution contained 5 mg. of albumin tagged with 250 to 500 microcuries of I^{131} per cc. The ionic (free) radioactive iodide was less than 1 per cent. The iodinated albumin was diluted with isotonic saline to yield a concentration of approximately 5 microcuries per cc. Ten cc. of this dilution was measured in a calibrated syringe and injected intravenously immediately following the injection of the T-1824 dye. The injected dose contained approximately 50 microcuries of I^{131} tagged albumin. Blood samples were obtained from the patient for determination of radioactivity simultaneously with the blood samples for determination of dye concentration. The plasma was separated by centrifugation. Five cc. aliquots of each plasma sample were counted with a Geiger counter and the counts per minute plotted against time. By extrapolation the counts per minute at zero time were determined (E). One cc. of the iodinated albumin solution containing approximately 5 microcuries per cc. was diluted volumetrically to 100 cc. with human plasma. A 5 cc. aliquot of this dilution was counted with a Geiger counter. This represented the standard (S) for the injected iodinated albumin. The plasma volume was then calculated from the following equation:

$$\text{Plasma volume (cc.)} = 100 \times \text{cc. of tagged albumin injected} \times \frac{S}{E}$$

All radioactivity measurements were made with a Tracer Lab. TGC-1 end window Geiger-Müller tube mounted in a lead chamber. The liquid plasma samples were counted in a cylindrical plastic cup with an internal diameter of 3 cm. The surface of the plasma sample was 1.5 cm.

[1] Published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[2] The T-1824 dye (lot 027021) used in this study was supplied by William R. Warner, Division of Warner-Hudnut, Inc., New York, N. Y.

from the end window of the Geiger tube. Five cc. plasma samples were found to be an infinitely thick layer in this counting cup, and radioactivity counts could be duplicated with an error of less than 2 per cent.

The experimental group consisted of 10 normal men, 7 patients with carcinoma and 5 patients with lymphoblastoma, 2 patients with duodenal ulcer, 2 patients with recent small pneumothorax, 1 patient with hypertension, and 1 patient convalescing from infectious hepatitis.

RESULTS

Comparison of the two methods

Plasma volumes were determined simultaneously with dye and iodinated albumin in 28 subjects (Table I). The mean plasma volume with iodinated albumin was 3255 cc. and with the dye method it was 3314 cc. The mean difference of 59 cc. was not significant ($t = 1.49$, $p > 0.1$). The correlation coefficient was 0.927 (Figure 1). An individual K factor for the dye was determined in 15 of these patients. The mean difference between the plasma volumes in this group as measured by the two methods was only 12 cc. A comparison was made of 28 plasma volumes calculated from the initial 10-minute plasma sample and the plasma volumes calculated from an extrapolation of 6 samples taken at 10-minute intervals for 1 hour (Table II). No significant difference between the volumes calculated from a single sample and multiple samples was found with either iodinated albumin or dye (iodinated albumin: $t = 1.0$, $p > 0.3$; dye: $t = 0.7$, $p > 0.5$). The standard deviation of the difference in the plasma volumes as calculated with the 10-minute sample and the multiple sample method was 91 cc. for both iodinated albumin and T-1824 dye.

Measurement of plasma volume change

In 10 patients the plasma volume was determined with both methods simultaneously, before and immediately after the rapid intravenous administration of 1000 cc. of 3.5 per cent polyvinylpyrrolidone (PVP), a synthetic plasma volume expander (Table III). The increase in plasma volume as measured by the two methods was not significantly different ($t = 0.4$, $p > 0.6$).

T-1824 dye and iodinated albumin disappearance rates

The decrease in plasma concentration of iodinated albumin and T-1824 dye during a 1-hour period after injection was compared (Table IV).

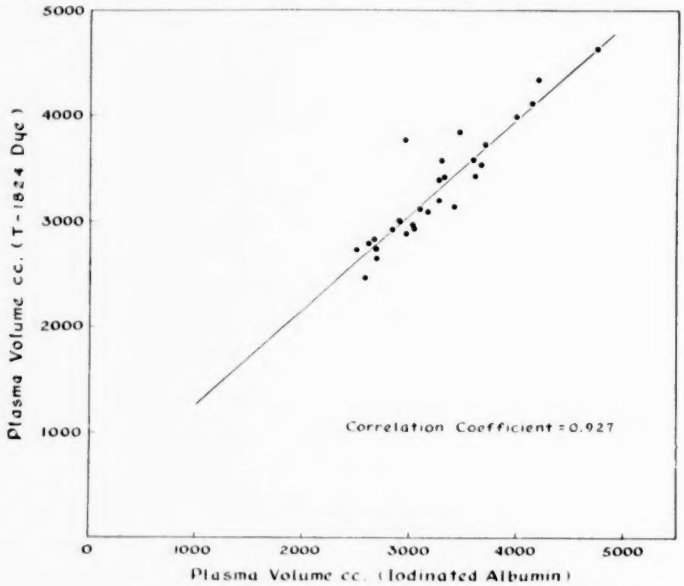

FIG. 1. CORRELATION BETWEEN PLASMA VOLUMES DETERMINED BY T-1824 DYE AND IODINATED ALBUMIN

TABLE I

Comparison of plasma volumes determined simultaneously with T-1824 dye and iodinated albumin

Patient	Plasma volume: T-1824 dye	Plasma volume: Iodinated albumin	Difference
	cc.	cc.	cc.
1	2937	2821	+116
2	3752	2940	+812
3	2647	2666	− 19
4	3114	3080	+ 34
5	3572	3287	+285
6	3423	3607	−184
7	3845	3450	+395
8	2724	2498	+226
9	3418	3312	+106
10	2971	3015	− 44
11	2935	3043	−108
12	4101	4137	− 36
13	4636	4747	−111
14*	3539	3651	−112
15*	3718	3701	+ 17
16*	3981	3982	− 1
17*	4343	4180	+163
18*	3395	3260	+135
19*	3144	3401	−257
20*	2836	2658	+178
21*	3091	3154	− 63
22*	3579	3580	− 1
23*	2895	2956	− 61
24*	3200	3260	− 60
25*	2791	2602	+189
26*	2455	2587	−132
27*	3007	2898	+109
28*	2743	2675	+ 68
Mean	3314	3255	+ 59

* Individual K factor determined.

TABLE II

Comparison of plasma volume calculated from 10-minute value and extrapolated value

Patient	T-1824 dye: Extrapolated value	T-1824 dye: 10-minute value	T-1824 dye: Diff.	Iodinated albumin: Extrapolated value	Iodinated albumin: 10-minute value	Iodinated albumin: Diff.
	cc.	cc.	cc.	cc.	cc.	cc.
1	2937	2894	− 43	2821	2747	− 74
2	3752	3627	−125	2940	3041	+101
3	2647	2741	+ 94	2666	2778	+112
4	3114	3154	+ 40	3080	3196	+116
5	3572	3481	− 91	3287	3542	+255
6	3423	3481	+ 58	3607	3698	+ 91
7	3845	3895	+ 50	3450	3607	+157
8	2724	2850	+126	2498	2538	+ 40
9	3418	3478	+ 60	3312	3259	− 53
10	2971	2891	− 80	3015	2928	− 87
11	2935	3029	+ 94	3043	3129	+ 86
12	4101	4186	+ 85	4137	4143	+ 6
13	4636	4745	+109	4747	4718	− 29
14	3539	3614	+ 75	3651	3592	− 59
15	3718	3687	− 31	3701	3655	− 46
16	3981	4067	+ 86	3982	4048	+ 66
17	4343	4356	+ 13	4180	4301	+121
18	3395	3171	−224	3260	3274	+ 14
19	3144	3196	+ 52	3401	3348	− 53
20	2836	2778	− 58	2658	2672	+ 14
21	3091	3057	− 34	3154	2983	−171
22	3579	3363	−216	3580	3473	−107
23	2895	2942	+ 47	2956	2892	− 64
24	3200	3263	+ 63	3260	3277	+ 17
25	2791	2850	+ 59	2602	2610	+ 8
26	2455	2502	+ 47	2587	2572	− 15
27	3007	3056	+ 49	2898	2940	+ 42
28	2743	2768	+ 25	2675	2676	+ 1
Mean	3314	3326	+ 13.5	3255	3273	+ 17.5

The mean per cent change in 1 hour was 11.5 for iodinated albumin and 11.0 for the dye. The difference between the means was not significant.

The urinary excretion of iodinated albumin was studied in 15 subjects. Over a period of two hours the urine contained 0.87 per cent of the injected radioactivity. In 4 patients 3.6 per cent of the injected radioactivity was excreted in the urine during an 8-hour period.

Thoracic duct studies

The cervical portion of the thoracic duct was cannulated in a 27 year old man undergoing a radical neck dissection for localized lymphoblastoma. The duct was not obstructed, and there was a free flow of lymph at the rate of approximately 1 cc. per minute. Samples of lymph and plasma were obtained at 10 to 20 minute intervals over a period of 140 minutes after the intravenous injection of 350 microcuries of I^{131} tagged albumin. The plasma and lymph concentrations of iodinated albumin, the ratios of these concentrations, and the per cent of the injected iodinated albumin remaining in the plasma at the various time intervals are shown in Table V. The radioactivity of cor-

TABLE III

Measurement of plasma volume with T-1824 dye and iodinated albumin before and after 1000 cc. polyvinylpyrrolidone (PVP)

Patient	T-1824 dye: Before	T-1824 dye: After	T-1824 dye: Diff.	Iodinated albumin: Before	Iodinated albumin: After	Iodinated albumin: Diff.
	cc.	cc.	cc.	cc.	cc.	cc.
6	3423	4224	801	3607	4371	764
7	3845	4403	558	3450	3872	422
8	2724	3421	697	2498	3480	982
9	3418	4067	649	3312	4085	773
10	2971	3695	724	3015	3737	722
11	2935	3686	751	3043	3809	766
12	4101	4853	752	4137	5068	931
13	4636	5432	796	4747	5604	857
15*	3718	4458	740	3701	4384	683
17*	4343	5067	724	4180	4722	542
Mean	3611	4330	719	3569	4313	744
S.D.	709	648	72	659	662	168

* Individual K factor determined.

TABLE IV

Per cent change in concentration of T-1824 dye and iodinated albumin in plasma in 1 hour

Patient	T-1824 dye	Iodinated albumin	Difference
	%	%	%
1	13.9	7.3	+ 6.6
2	2.6	20.0	−17.4
3	17.1	19.7	− 2.6
4	13.3	18.6	− 5.3
5	8.3	15.5	− 7.2
6	10.8	18.3	− 7.5
7	7.9	21.7	−13.8
8	24.1	19.5	+ 4.6
9	9.9	11.1	− 1.2
10	9.0	8.9	+ 0.1
11	12.3	12.7	− 0.4
12	13.2	11.7	+ 1.5
13	13.4	8.1	+ 5.3
14	11.3	6.1	+ 5.2
15	15.6	10.2	+ 5.4
16	12.9	8.5	+ 4.4
17	13.1	11.9	+ 1.2
18	5.4	13.4	− 8.0
19	12.4	8.4	+ 4.0
20	6.0	7.6	− 1.6
21	7.0	3.9	+ 3.1
22	5.8	5.5	+ 0.3
23	9.5	8.6	+ 0.9
24	11.4	7.7	+ 3.7
25	10.5	9.3	+ 1.2
26	13.6	8.6	+ 5.0
27	10.1	11.5	− 1.4
28	7.9	8.5	− 0.6
Mean	11.0	11.5	− 0.5

TABLE V

Studies on human thoracic duct lymph after the intravenous injection of iodinated human serum albumin

Minutes after injection	I^{131} dose per 100 cc.		Plasma/lymph ratio	Dose in plasma
	Plasma	Lymph		
	%	%		%
10	2.80	0.07	40.0	98.3
20	2.65	0.10	26.5	91.3
30	2.57	0.18	14.3	90.2
45	2.56	0.35	7.3	89.9
65	2.60	0.87	3.0	91.3
85	2.43	0.90	2.7	85.0
105	2.49	1.00	2.5	87.4
135	2.27	1.08	2.1	79.4

responding plasma and lymph samples is graphically illustrated in Figure 2. The plasma protein concentration was 8 gm. per 100 cc. with 5.3 gm. albumin; the lymph protein concentration was 5.7 gm. per 100 cc. with 4.2 gm. albumin. During the 6-hour period following the injection of the iodinated albumin, 4.1 per cent of the injected radioactivity was excreted in the urine. One hour after intravenous injection, approximately 10 per cent of the iodinated albumin had disappeared from the plasma. The plasma volume was 3512 cc. in this patient as measured by the iodinated albumin method.

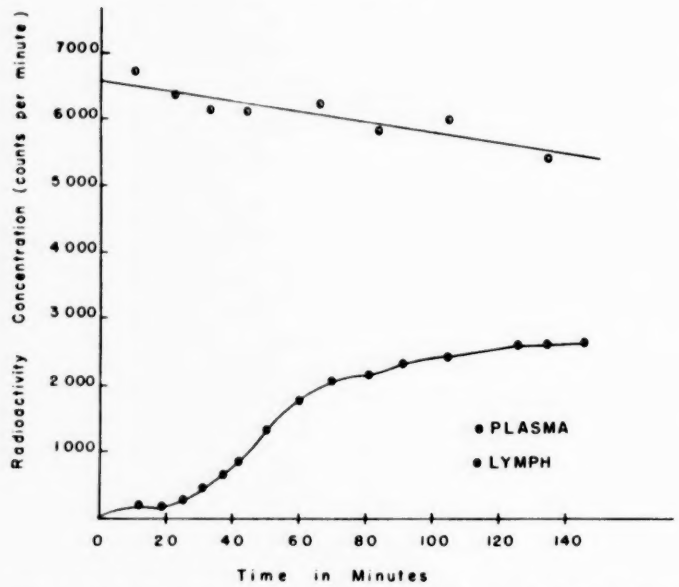

FIG. 2. RADIOACTIVITY CONCENTRATION IN 5 CC. SAMPLES OF PLASMA AND THORACIC DUCT LYMPH FOLLOWING INTRAVENOUS INJECTION OF I^{131} TAGGED SERUM ALBUMIN

DISCUSSION

Sterling (7), and Fine and Seligman (8) have established the reliability of the combination of I^{131} to albumin by *in vitro* and *in vivo* studies. Human serum albumin tagged with I^{131} would appear to fulfill all the desired requirements for plasma volume measurement based on the tagged protein dilution principle. Therefore, a careful comparison of the T-1824 dye and the iodinated albumin methods should be a logical and reliable experiment to study the validity of the dye method. Storaasli and his co-workers (1) and Aust and his co-workers (2) have reported that plasma volumes measured with T-1824 dye are greater than those determined simultaneously with iodinated albumin. These authors suggested that a certain amount of dye escapes from the vascular system during the first few minutes after injection. Crispell and his co-workers (3), however, found no significant difference in plasma volumes as measured by the two methods. In the present study, the T-1824 dye and the iodinated albumin methods of plasma volume measurement did not give significantly different results. This is to be expected since several investigators (9–11) have demonstrated that the intravenous injection of T-1824 dye is followed by a rapid combination with plasma albumin. It is very unlikely that any dye is lost before binding since the dye is bound to protein very rapidly. An error in the determination of the K factor in the dye method will introduce a systematic error in the calculation of the plasma volume. Such an error may account for the reported discrepancy between the two methods. From the results of the present study, the concept that there is a rapid loss of T-1824 dye from the circulation after injection and before binding to plasma protein appears untenable.

Studies on the rate of disappearance of iodinated albumin in the thoracic duct lymph of a human subject revealed that very little iodinated albumin appeared in the thoracic duct lymph during the first 30 minutes after intravenous injection. In experimental animals a somewhat larger amount of tagged protein appears in the thoracic duct lymph during the first hour after injection (12–17). From the present study it would seem that in the human the amount of tagged albumin appearing in thoracic duct lymph during the period of determination of the plasma volume is small and does not significantly influence the measurement of plasma volume.

Blood volume measurements with tagged erythrocytes have been reported to result in lower values when compared with blood volumes determined by tagged plasma protein methods (18–24). This discrepancy has been attributed to the fact that the total body hematocrit is lower than the venous hematocrit. Gibson and his associates (23) studied the hematocrit of the small blood vessels using I^{131} tagged albumin and erythrocytes tagged with Fe^{59}. The hematocrit of the blood in the small vessels was found to be much lower than the hematocrit of arterial blood in all organs except the spleen. These studies assume a rigid boundary to the vascular system. The tagged protein methods measure a rapidly exchangeable pool of albumin. The anatomic limits of this albumin pool may not be identical with the anatomic limits of distribution of the erythrocytes. In a strict sense the tagged protein methods should be used as a measure of this pool of albumin. Making the assumption that all the albumin in the pool is contained in the plasma volume and that the concentration of the albumin is constant throughout the plasma, one can calculate the plasma volume. Unfortunately these assumptions are not entirely correct. Determination of the plasma volume by methods employing tagged erythrocytes is based on the assumption that the ratio of plasma to erythrocytes is constant throughout the body. This assumption is also not entirely correct. Thus, the definition of plasma volume, like the definition of extracellular fluid, will always depend upon the method used in its measurement.

Plasma volume measurements made with T-1824 dye are as valid and reliable as those made with iodinated albumin. When carefully done the two methods give similar results. The criticisms leveled at the dye method can also be made of any tagged protein method for plasma volume measurement.

SUMMARY AND CONCLUSIONS

1. The T-1824 dye and the iodinated albumin methods for plasma volume measurement when performed simultaneously in human subjects gave values which did not differ significantly.

2. When the plasma volume was expanded by polyvinylpyrrolidone (PVP), the plasma volume change as measured by T-1824 dye and by iodinated albumin did not differ significantly.

3. Studies of human thoracic duct lymph with iodinated albumin revealed that very little tagged albumin appeared in the lymph during the first half hour after intravenous injection.

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ELECTROLYTE EQUILIBRIA IN ERYTHROCYTES DURING DIABETIC ACIDOSIS [1, 2]

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It has been suggested that changes in the electrolyte composition of the erythrocyte may parallel such changes in other body cells (1). The main objections to this theory have been that the red cell has not only a slower metabolic rate (2) but also an anion pattern that differs markedly from that of other cells (3). In the studies here reported, erythrocyte base and water were measured during diabetic acidosis and recovery, and an attempt made to interpret the observed data in terms of a logical and orderly sequence of events, both within the erythrocyte and within other body compartments. The shifts found in base and water were qualitatively similar to those demonstrated previously by other workers in total intracellular base and water, using balance techniques (4–6). Furthermore, in most instances they were quantitatively reconcilable with changes that have been demonstrated in the anion composition of the erythrocyte (7). This suggests that this readily available cell may serve as an indicator of intracellular base and water shifts in disease.

METHODS

Blood samples were drawn from patients admitted in severe diabetic acidosis before the institution of any therapy, at 6 and 12 hours after the beginning of therapy, and at 24-hour intervals thereafter until the patient's discharge from the hospital. Blood was collected in 20-ml. syringes containing 0.02 ml. of heparin ("Liquaemin," Hoffmann-LaRoche, sodium content 0.0035 mEq./20 ml. of blood). Measurements of water, sodium, potassium, and hematocrit were made on whole blood. Plasma was separated immediately for the determination of pH, water, sodium, potassium, and chloride.

Intake and urinary output of water, sodium, potassium and chloride were measured on all patients for the first 24 hours. The urinary excretion of these elements was measured throughout the patient's hospital stay.

The water content of whole blood and plasma was determined by delivering 1 ml. into a tared bottle, weighing and drying to constant weight.

Chlorides were done by the method of Schales and Schales (8).

Sodium and potassium were measured photometrically by an internal standard flame photometer (9) built by the authors. Standards were compounded to approximate the levels of interfering ions encountered in the material being analyzed. Recovery experiments for both plasma and whole blood showed the instrument to have an accuracy of ± 1 per cent for sodium and ± 1.5 per cent for potassium. Plasma was analyzed without precipitation of protein. Whole blood was prepared for analysis by a modified Neumann wet-ash procedure (10).

Plasma pH was measured with a Beckman model G pH meter.

Hematocrit determinations were done in duplicate on carefully mixed blood. The tubes were spun for 45 minutes at 3000 RPM in a centrifuge with a head radius of 16 cm. They were respun for successive 15-minute periods until a constant value was obtained. Readings were made to the bottom of the buffy coat and a correction factor for trapped plasma of 0.98 applied (11, 12). Duplicate hematocrit determinations checked within 0.5 per cent.

Intracellular sodium, potassium and water were calculated by the formula (10):

$$C_e = \frac{C_b - C_p(1 - V)}{V}$$

in which

C_e = concentration in cells in mEq./L.
C_b = concentration in whole blood in mEq./L.
C_p = concentration in plasma in mEq./L.
V = corrected hematocrit expressed as a decimal.

Values for sodium and potassium per kilogram of water were calculated from the formula:

$$C/Kg\ H_2O = \frac{C_L \times 1000}{Gm.\ H_2O}$$

where

$C/Kg\ H_2O$ = concentration per kilogram of water
C_L = concentration per liter of substance
$Gm.\ H_2O$ = grams of water per liter of substance.

RESULTS

Table I presents the mean results on 21 normal individuals whose blood was analyzed to test the

[1] This work was aided by a grant from The Diabetic Fund.

[2] Presented in abstract form at the 43rd annual meeting of the American Society for Clinical Investigation, Atlantic City, N. J., April 30, 1951.

TABLE I

Plasma and red cell Na and K in 21 normal human subjects

	Sodium				H_2O in Gm./L		HCT.	Potassium			
	Plasma		Red cells		Plasma	Red cells	Corr.	Plasma		Red cells	
	mEq./L	*mEq./ Kg H_2O*	*mEq./L*	*mEq./ Kg H_2O*			f = .98	*mEq./L*	*mEq./ Kg H_2O*	*mEq./L*	*mEq./ Kg H_2O*
Average	139.8	150.0	16.3	21.5	927	721	43.8	4.0	4.31	104.5	146.0
S.D.	2.5	3.1	3.6	5.0	15.7	17.3	3.3			6.4	7.9

reproducibility of the methods employed; and to obtain a set of values for comparison with those obtained in diabetic acidosis and recovery.

Table II shows the results obtained in 9 patients during severe diabetic acidosis and for several days following recovery. Fifteen patients were studied but 6 are excluded from this report; one because of death four hours after admission, one because of inadequate blood samples, and four because of evidence of long-standing renal damage. In the 9 remaining patients, analyses of the urine and of the blood non-protein nitrogen on recovery from acidosis gave normal results. All these patients, as can be seen from Table II, presented data which, while differing quantitatively, showed uniform qualitative changes. The variations from patient to patient are undoubtedly the outcome of differences in the duration and cause of the acidosis in each case.

Table III shows the mean results of the determinations on the 9 patients on admission to the hospital in severe acidosis, following 6 to 12 hours of therapy, following 36 hours of therapy, and on discharge from the hospital. Values for 6 to 12 hours are averaged, since some patients showed maximum changes at 6 and others at 12 hours, as can be seen from Table II.

The changes in plasma electrolytes and water in these patients on admission to the hospital in severe diabetic acidosis were similar to those found by other investigators (4–6, 13–15) and were in keeping with a loss of water due to glucose diuresis and a parallel loss of fixed base. Plasma dehydration was demonstrated by a fall in plasma water from 930 to 910 Gm./L., while a normal concentration of sodium per kilogram of plasma water reflected the parallel loss of base. Since this base was excreted largely in combination with organic acids a relative excess of chloride would be expected in the plasma and this was found. The average chloride : sodium ratio in the 9 patients on admission was 0.86 (Table II) in contrast with the normal ratio of 0.74. The mean concentration of potassium in the plasma water was slightly elevated, and was highest in those patients in whom there had been a decreasing urine output in the hours preceding admission. This suggests that high levels of serum potassium in untreated diabetic acidosis are due to failure of the kidney (embarrassed by low blood pressure and dehydration) to clear the large amounts of potassium being released from the cells.

During the first 12 hours of treatment all patients received large amounts of insulin and 0.85 per cent sodium chloride solution administered parenterally. The values for plasma water and sodium at 6 to 12 hours demonstrate the efficiency of the intravenous administration of salt solution in combating dehydration. Plasma water was normal at 12 hours, and despite the large amounts of fluid administered (up to 8 liters) no patient showed chemical or clinical evidence of overhydration. The mean plasma sodium value was slightly elevated at 12 hours. Two patients exhibited hypernatremia, which slowly subsided. One (E. G.) had an initial plasma sodium of 165 mEq./Kg H_2O; the other had a normal plasma sodium concentration initially which rose to 168 mEq./Kg H_2O during treatment. One undesirable effect of 0.85 per cent sodium chloride solution in the treatment of this condition was seen in the accentuation, in all cases, of the already existing hyperchloremia. It is not possible to say how much this perpetuated the acidosis. However, plasma pH and CO_2 had returned to normal in all cases by 36 hours (between 18 and 24 hours in most cases) after admission so that the acidotic effect, if any, was not of long duration in these patients with adequate renal function.

The concentration of plasma potassium decreased an average of 20 per cent in all patients in the first 12 hours of treatment. This has been de-

scribed by many investigators (6, 14–16) and does not correlate with changes in blood sugar, plasma bicarbonate or pH. Many explanations have been offered for this decrease, including dilution of extracellular fluid, intracellular shifts and urinary losses. Since no measurements of extracellular volume were made on these patients, no exact figures for total extracellular potassium are

TABLE II

Analytical data on nine patients during recovery from diabetic coma

Patient	Time from start of treatment	pH	CO_2 mM/L	Blood sugar mgm. %	HCT. (corr.)	Plasma Na mEq./Kg H_2O	Plasma K mEq./Kg H_2O	Plasma Cl mEq./Kg H_2O	Plasma H_2O Gm./Kg	RBC Na mEq./Kg H_2O	RBC K mEq./Kg H_2O	RBC H_2O Gm./Kg
	0 hours	—	8	805	47.4	140	6.0	110	915	50.0	129	724
A. B.	6	—	20	400	38.6	145	3.8	125	937	13.0	108	692
65	12	—	24	325	36.8	154	4.3	127	937	0	136	649
	36	—	—	82	39.1	140	4.6	114	929	13.0	119	738
	3 days	—	21	211	42.8	145	4.6	—	930	6.8	102	740
	2 hours*	7.25	6	292	52.9	156	3.8	128	861	20.5	123	735
B. F.	6	7.50	11	290	43.1	146	3.4	127	918	26.0	128	705
19	12	7.49	17	275	42.7	152	2.8	135	922	9.0	126	719
	36	7.49	29	87	41.2	151	2.8	121	930	11.3	134	709
	4 days	7.40	23	143	40.1	148	3.4	107	927	7.5	145	691
	0 hours	7.02	8	515	57.9	165	4.4	141	910	—	—	—
	6	7.05	—	400	37.8	173	3.2	160	930	11.0	146	702
E. G.	12	7.20	17	—	33.5	178	3.1	166	931	2.6	143	690
35	36	7.48	27	157	30.7	172	3.3	157	941	18.4	158	658
	60	7.45	—	—	31.6	157	3.7	137	937	11.5	131	690
	3 days	—	—	—	33.8	150	4.7	128	933	27.2	134	728
	5	7.40	21	257	33.3	147	4.5	131	932	27.9	140	709
	0 hours	7.15	7	417	51.9	149	6.1	129	902	4.3	128	729
H. H.	6	7.40	7	363	41.9	149	4.7	140	926	0	137	707
21	12	7.42	11	183	41.0	143	3.6	137	936	13.6	122	720
	48	7.53	21	106	41.6	144	3.9	128	922	7.0	134	678
	3 days	7.42	—	—	40.0	144	4.2	129	927	21.3	122	737
	0 hours	—	4	1160	45.7	140	7.6	120	916	12.5	110	750
	6	—	5	750	39.3	158	3.2	144	926	20.1	118	736
E. P.	12	—	14	255	36.1	168	3.3	147	928	1.2	139	645
18	36	—	23	280	33.5	158	3.3	128	932	9.4	136	699
	5 days	—	—	383	38.1	155	3.5	116	931	0	135	710
	9	—	22	190	38.3	150	4.3	120	925	14.7	132	742
	0 hours	7.00	8	383	48.2	142	5.2	127	911	26.2	116	730
R. P.	6	7.20	17	186	38.7	150	3.1	141	927	0	155	685
49	20	7.36	20	230	40.2	141	3.9	127	935	4.0	142	649
	48	7.30	—	373	43.6	145	4.2	126	929	22.7	134	697
	6 days	7.41	21		40.6	150	4.4	127	925	22.5	144	703
	0 hours	—	10	475	48.5	141	6.0	—	916	25.0	145	707
M. P.	6	7.35	16	162	44.5	139	3.9	—	935	37.5	150	714
59	12	—	22	30	43.1	149	3.9	—	927	27.0	147	720
	3 days	7.38	20	109	48.3	141	3.2	—	925	14.0	141	714
	0 hours	7.14	5	271	46.2	146	4.4	127	916	21.5	130	695
	6	7.25	13	122	33.6	148	2.4	138	944	13.9	123	643
B. R.	12	7.40	22	160	34.2	147	2.6	138	940	14.2	117	658
16	5 days	—	29	264	33.9	149	2.8	116	930	21.0	148	666
	7		29	256	34.1	153	3.7	119	930	28.1	142	652
	11	7.36	27	90	37.2	152	4.4	122	932	30.0	139	697
	0 hours	6.80	6	350	46.4	153	5.0	141	905	11.5	123	748
	6	—	6	154	38.1	157	3.8	162	925	0	133	728
J. R.	12	7.30	10	154	38.5	154	2.8	145	930	14.7	127	727
37	36	—	20	182	31.4	144	3.0	134	933	33.0	124	716
	3 days	—	23	104	32.9	149	2.6	129	929	—	130	736
	7	7.41	—	—	31.3	148	3.8	116	925	28.0	138	754

* This patient had received 60 units of insulin 2 hours before the first blood sample was drawn.

TABLE III

Plasma and red cell Na and K in 9 diabetic subjects

	Sodium				H_2O in Gm./L		Potassium			
	Plasma		Red cells		Plasma	Red cells	Plasma		Red cells	
	mEq./L	*mEq./Kg H_2O*	*mEq./L*	*mEq./Kg H_2O*			*mEq./L*	*mEq./Kg H_2O*	*mEq./L*	*mEq./Kg H_2O*
Admission	135	148	15.3	21	910	728	5.0	5.5	89	123
6 and 12 hours	144	154	6.5	9.3	930	689	3.2	3.4	91	130
36 hours	140	150	9.6	14.0	931	688	3.2	3.5	92	132
Discharge	138	148	14.4	19.8	928	712	3.8	4.1	96	134

available. However, assuming an extracellular volume of 30 per cent of body weight at 12 hours and estimating the initial extracellular volume by correcting the 12-hours volume for the plasma water change between admission and 12 hours, and multiplying each value by the appropriate potassium concentration, an approximation of the total extracellular potassium change may be obtained. Using this calculation and the average values from all 9 patients, the mean loss of extracellular potassium was 33 mM—approximately one-third of the initial total extracellular content of 93 mM—whereas the average negative balance of potassium was 35 mM during this 12-hour period. Thus the urine contained all the potassium lost from the extracellular space during this period of treatment.

This loss of potassium from the body at a time when there is a large demand for the ion due to the deposition of glycogen and protein in cells may be due to failure of the renal mechanism to change rapidly from a state in which large amounts of potassium must be excreted to one in which the ion must be conserved. On the other hand, in the absence of any specific metabolic dysfunction which disturbs this relationship, the plasma potassium concentration may be related to the total body content of this ion. Since these patients had a low total body potassium (4, 15), and since insulin had restored their metabolic processes towards normal, the kidney may have been excreting potassium during these hours to restore the normal potassium ratio between plasma and cells. In defense of this hypothesis was the slow and parallel rise in serum and erythrocyte potassium concentrations during the later phases of recovery.

No potassium was given to any of these patients parenterally, but fluids or foods high in potassium were given orally as soon as tolerated. This was possible in all cases between 12 and 18 hours after the start of therapy. No patient exhibited signs or symptoms characteristic of hypopotassemia at any time nor were electrocardiograms during recovery characteristic of this syndrome. Plasma potassium concentrations rose slowly until at discharge they approached the normal value of 4.3 mEq./Kg H_2O. Values for plasma water and sodium remained normal (with the exception of 2 cases of hypernatremia) from 12 hours after hospital admission until discharge.

On admission, prior to any therapy, the red cells had a mean deficit of potassium of 23 mEq./Kg of cell water, 16 per cent less than the normal. This deficit is comparable to that which occurred in the first patient reported by Atchley and his associates (4), a patient in whom 13 per cent of the estimated total body potassium was lost during the induction of experimental diabetic acidosis. However, the water content of the red cells was normal, despite loss of potassium, dehydration of the surrounding plasma, and the obviously dehydrated state of the patient. The mean cell sodium concentration appears normal in Table III but if one excludes a single value of 50 mEq./Kg H_2O, the mean was 16.1 mEq./Kg of cell water, or 20 per cent below the normal value, confirming the findings of Danowski and his co-workers (13).

Within the first 12 hours of treatment the red-cell potassium values increased from 123 to 130 mEq./Kg H_2O. However, this change could be entirely accounted for by the loss of water from the cells and did not indicate an intracellular shift of this ion since, despite the dehydration of the extracellular fluid, the mean red-cell water content decreased during this period from a normal of 728 to 689 Gm./L. Sodium values fell to an average of 55 per cent of the admission value, and in 6 of the 9 patients reached 0 at either 6 or 12 hours.

At 36 hours the potassium concentration had begun to rise slightly. However, the increase of

2 mEq./Kg H_2O which had occurred was far smaller than the potential exchange of 1.5–2 mEq. of potassium per liter of red cells per hour which has been demonstrated *in vitro* (2). Whether this slow accumulation of potassium in the erythrocyte is due to competition for the ion with other cells having a more active glycogen metabolism or whether it is related to the reaccumulation of water and phosphate in the cell (17) cannot be ascertained from the data on these patients. Red-cell water at 36 hours remained decreased. Red-cell sodium had increased and was returning to normal more rapidly than potassium.

At the time of the patient's discharge from the hospital, total red-cell potassium had increased considerably, although the increase in cell water, which had risen to a mean value of 712 Gm./L, masked much of this rise. Only two patients left the hospital with a normal concentration of red-cell potassium. There was a direct correlation between the level of potassium and the duration of the patient's hospital stay. Red-cell sodium, on discharge, had almost reached the normal concentration of 21 mEq./Kg of cell water, having a mean value of 19.8 mEq./Kg H_2O.

DISCUSSION

The data in Table I show that there is a higher total base concentration in the red cells than in the plasma in keeping with the normal cation distribution between all cells and their surrounding media (3, 18). However, Table III shows that in diabetic acidosis and in the post-acidotic state this distribution is reversed and the total base concentration in the red cells is lower than in the surrounding plasma. This difference requires examination in light of the need for osmotic equality between cells and plasma, as well as the need for electroneutrality in each phase. Knowledge of the concentrations of anions as well as cations in the red cell is required. Since no measurements of erythrocyte anions were made on these patients, it was necessary to turn to the literature for these data in order to explain the changes observed.

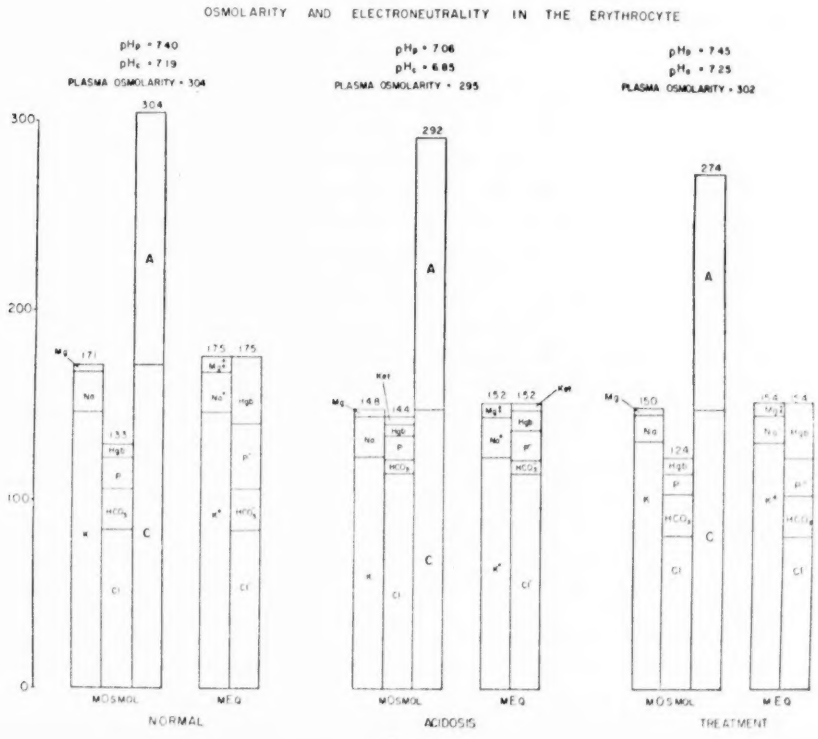

FIG. 1

Figure 1 has been constructed according to the manner of Gamble (3) to demonstrate the electrolyte composition of the red cell water in the normal, the diabetic in acidosis, and the diabetic 36 hours after treatment for acidosis. In the left hand columns of each section the ions are plotted as millimols and the total osmolarity of the cell water is shown by superimposing total anions on total cations. In the right hand columns the ions are plotted in terms of milliequivalents to demonstrate electroneutrality. Concomitant values for plasma pH and osmolarity and cell pH are shown above each diagram. The values for cell sodium and potassium and plasma pH are from Tables I and III. The hemoglobin has been assumed to be 300 Gm./L of red cells with a molecular weight of 67,000. All other values are from the literature (3, 7, 13, 14, 19–28). While the application of such derived data, obtained in part from studies of normal cells, in conjunction with actual analytical data is open to criticism on obvious grounds, its use seems justified since it explains the changes observed in a manner consistent with the fundamental concepts of osmotic equality and electroneutrality.

It can be seen from these diagrams that electroneutrality is maintained at all times within the cell, despite the large changes in cell base. This is made possible by changes in valency and concentration among the anions, notably hemoglobin and phosphate, in response to changes in cell pH (7, 19, 23, 24). Osmotic equality obtains in the normal and the diabetic in acidosis. In the latter, the replacement of polyvalent anions by univalent chloride—a change caused by the reduction in cell pH—accounted for the normal total osmolarity of the cells and provided an explanation for the normal water content of the cells at this time. Thirty-six hours after the start of therapy both total cations and anions were reduced since neither potassium nor phosphate had yet reaccumulated in the cells, but, with the return of pH towards normal, chloride had left the cell. The total osmolarity of the cell water was then only 274 as compared with a plasma osmolarity of 302.

There is a definite mathematical relationship between this reduced osmolarity and the observed fall in erythrocyte water. Total osmolarity fell from 292 to 274 (6 per cent decrease) and water fell from 728 to 688 Gm./L (5.5 per cent decrease). However, to accept this value for cellular osmolarity we must assume either an unstable state or osmotic inequality between two contiguous phases of body water. The rapid diffusibility of water across cell membranes makes the existence of an unstable state unlikely, while the magnitude of the pressure implied by a difference of 26 milliosmols (500 mm. of mercury) makes a state of osmotic inequality equally unreasonable. It is more likely that knowledge of the osmotically active components of abnormal red cells is incomplete and that organic compounds exist within the cell at this time which account for the discrepancy observed. These compounds must be electrically neutral or equally divided between anions and cations if electroneutrality is to be preserved.

The low potassium values observed in the erythrocytes of these patients on admission were consistent with the available indirect evidence of low intracellular potassium (4–6). Balance data usually reveal a continuing small loss of potassium during the early hours of treatment (5, 6), but the data reported here indicate that, at least within the erythrocyte, there was a prompt cessation of potassium loss on the initiation of therapy. The subsequent slow rise in red cell potassium paralleled calculated rises in total intracellular potassium (4–6, 15).

Fewer data are available on the movements of sodium in and out of cells, and no clear concept of the nature or causes of these movements has yet been advanced. However, 7 of the 8 patients of Danowski and his associates (5), studied by balance technique, showed a marked loss of intracellular sodium during the 22 to 37 hour period following the beginning of therapy. The difference in time between this loss and our findings may be due to a delay in renal excretion, a difference in the time of discharge of sodium between the erythrocyte and other body cells, or the administration of glucose during the preceding period. Nine of the ten patients presented by Seldin and Tarail (6) showed negative intracellular balance of sodium during one or more periods of study and 8 presented this finding in the first 7 hours of treatment.

Correlation of shifts in total intracellular water with shifts observed in red cell water was also significant. Calculation of the balances of intracel-

lular water from the data of the two groups of investigators referred to above (5, 6) [3] revealed that, in most instances, there was a transient period of loss of water from the total intracellular mass during the early hours of treatment. Six of Danowski's patients showed this at some time during the first 37 hours of treatment, and in the 10 patients of Seldin and Tarail all but one lost intracellular water at some time during the first 5 hours of treatment and the one remaining patient showed this by 17 hours.

This correlation between changes in total intracellular base and water and changes in these elements within the erythrocytes appears to be more than fortuitous, particularly in view of the different therapeutic measures employed in the three groups of patients. Our patients received only insulin and saline. Danowski's group received glucose and large amounts of potassium in addition to insulin and saline, and Seldin and Tarail's patients received insulin, saline, and small to moderate amounts of glucose.

SUMMARY

1. Values for plasma and erythrocyte sodium, potassium, and water in 9 patients during diabetic acidosis and recovery are reported. For comparison, values in 21 normal subjects are presented.

2. On admission in diabetic acidosis, plasma base concentration was normal and plasma water was decreased. Erythrocyte sodium and potassium concentrations were decreased while the water content was normal.

3. Following therapy with insulin and 0.85 per cent sodium chloride solution, the patients showed rapid clinical improvement with return of plasma pH, bicarbonate and water to normal. Undesirable side effects of therapy were occasional hypernatremia, the constant occurrence of transient hyperchloremia and hypokalemia persisting for several days.

4. With treatment, the erythrocytes lost no further potassium, but did lose both water and sodium. Sodium reaccumulated in the cells more rapidly than potassium or water. In most patients both cell water and potassium were lower than normal at the time of discharge from the hospital.

5. The mechanism of these changes is discussed.

6. There is a parallelism between water and base shifts in the erythrocytes and in the total cell mass during recovery from diabetic acidosis.

[3] (5) Calculated from the data of Tables I and III.
(6) Calculated from the data of Tables I, II and III.

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OBSERVATIONS CONCERNING THE CHOLATE: CHOLESTEROL RELATIONSHIP IN CLINICAL AND EXPERIMENTAL NEPHROSIS [1, 2]

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(Submitted for publication August 13, 1952; accepted November 14, 1952)

The occurrence of hypercholesteremia in the nephrotic state is a long recognized phenomenon (1), but its mechanism remains to be determined. Apparently the hypercholesteremia cannot be ascribed to a primary "lipoid degeneration" of the kidney (2).

Recently, studies from this laboratory (3–6) have indicated that there is a marked derangement of cholate metabolism in certain clinical and experimental states of hypercholesteremia. It therefore was considered of interest to investigate the metabolism of this particular steroid in both clinical and experimental nephrosis.

I. *The Cholate: Cholesterol Relationship in Clinical Nephrosis*

(1) *Methods*

Samples of blood serum were obtained from 18 patients suffering from nephrosis for periods of time varying from one to 24 months. There were 12 males and 6 females, varying in age from 3 to 65 years. "Lipoid" nephrosis was believed present in 11 instances, the nephrotic stage of chronic glomerulonephritis in six patients, and Kimmelstiel-Wilson syndrome with nephrosis in one case. Serum cholesterol and cholate concentrations were determined in each instance, according to technics previously described [3] (7, 8).

(2) *Results*

The cholate content of the serum invariably was elevated if the patients also were hypercholesteremic (see Table I). Thus, the serum cholesterol concentrations of 15 nephrotic patients with hypercholesteremia varied from 450 to 1060 mg. per 100 cc., and their serum cholates from 10.0 to 44.0 mg. per 100 cc. These values were in marked contrast to those obtained from 25 normal human subjects in whom the serum cholesterol ranged from 110 to 280 mg. per 100 cc., and the serum cholate from 1.7 to 7.2 mg. per 100 cc.

On the other hand, the serum cholate concentrations were found to be normal (1.0, 6.4, 5.0 mg. per 100 cc.) in three nephrotic patients with normal serum cholesterol levels (110, 250, 200 mg. per 100 cc., respectively). This finding of "hypercholatemia" in nephrotic patients only when they also exhibited hypercholesteremia prompted the following experimental investigations:

II. *The Cholate: Cholesterol Relationship in Experimental Nephrosis*

A. *The plasma cholesterol and cholate concentrations in rats with experimental nephrosis*

The studies of Heymann and Lund (9) have shown that the chronic disease produced in rats by the injection of rabbit anti-rat kidney serum closely simulates the nephrotic state as it is observed in human subjects. This method, therefore,

TABLE I

The average serum cholesterol and cholate concentration in nephrotic patients

Type of patient	No. of patients	Average plasma cholesterol (*mg./100 cc.*)	Average plasma cholate (*mg./100 cc.*)
Normal control	25	228	5.3
Range:		(110–280)	(1.7–7.2)
S.E. Mean:		±8.7	±0.26
Nephrotic (hypercholesteremic)	15	695	19.2
Range:		(450–1060)	(10.0–44.0)
S.E. Mean:		±45.0	±2.8
Nephrotic (normocholesteremic)	3	180	4.4
Range:		(110–250)	(1.0–6.4)

[1] Aided by grants from the American Heart Association, Monterey, California, Heart Association, and the United States Public Health Service.

[2] Presented in part at the May, 1952, meeting of the American Society for Clinical Investigation, Atlantic City, N. J.

[3] A small percentage of the result obtained by this technic may represent noncholate chromogen substances.

was utilized to study cholate metabolism in experimental nephrosis.

(1) *Methods*

Rabbit anti-rat kidney serum, prepared and sent to us by Dr. A. I. Knowlton (10), was injected intravenously into immature (4 to 8 weeks old) male rats (Long-Evans strain). One-third cubic centimeter of this serum was injected into each of 30 rats on each of two successive days. Renal disease, as indicated by the onset of marked albuminuria and hyperlipemia (9), developed within one week in all but two rats.

Plasma cholesterol and cholate concentrations were determined in 22 rats prior to, and one week following, the injections of anti-rat kidney serum. Similar analyses were obtained in a group of 10 normal control animals.

(2) *Results*

Both hypercholesteremia and hypercholatemia consistently were found (see Table II). The average plasma cholesterol of the 22 nephrotic rats rose from 71 mg. per 100 cc. (prior to the injection of immune serum) to 460 mg. per 100 cc., one week after the injections of anti-rat kidney serum. The average plasma cholate concentration of the same rats rose from 5.9 to 23.1 mg. per 100 cc.

B. *The rate of disappearance of cholate from the blood of nephrotic rats following its intravenous injection*

(1) *Methods*

In order to test the ability of the nephrotic rats to eliminate cholate from their blood, the following experiment was performed. Twenty-five milligrams of sodium cholate were injected intravenously into 11 of the above nephrotic rats and into 10 control animals. Nephrotic rats were selected whose plasma cholate levels were only moderately elevated at the time of the injection. In each instance samples of blood plasma were obtained at four different times; prior to, and immediately and one and two hours after the injection of sodium cholate.

(2) *Results*

The intravenous injection of cholate induced a considerable and comparable rise of plasma cholate in both the nephrotic and the control animals (see Table III). The excess cholate largely disappeared from the plasma of the control group of rats within one hour after its administration. It was eliminated entirely at the end of two hours. However, considerable elevation of plasma cholate was found in the nephrotic rats at the end of one hour and this persisted even two hours after the cholate injection. Thus, there appeared to be a delay in the disappearance of the injected cholate from the plasma of the nephrotic rats.

C. *The effect of cholate administration upon the plasma cholesterol of nephrotic rats*

(1) *Methods*

Heymann and Lund (9) observed that the initial hypercholesteremia which developed in nephrotic rats soon decreased to somewhat lower although still elevated levels. This finding prompted the following experiment: One hundred milligrams of cholate were fed daily by gastric intubation to 11 of 17 nephrotic rats (one week after the injection of immune serum). The same amount of cholate was given to 11 of 19 control animals. The plasma cholesterol was determined in each instance prior to the feeding of cholate and again five days later. Similar determinations were obtained in the remaining nephrotic and control rats that did *not* receive cholate feedings. All rats were fed the stock laboratory ration.

(2) *Results*

The elevated plasma cholesterol levels of the 11 nephrotic rats to which cholate was fed, were maintained; their average plasma cholesterol rose from 495 mg. per 100 cc. to 508 mg. per 100 cc. (see Table IV). On the other hand, the average plasma cholesterol of the six nephrotic rats *not* receiving

TABLE II

Average plasma cholesterol and cholate concentration of nephrotic rats

Type of rat	No. of rats	Average weight *gm.*	Average plasma concentration (*mg./100 cc.*) Before injection of immune serum		After injection of immune serum (1 week)	
			Cholesterol	Cholate	Cholesterol	Cholate
Nephrotic	22	126	71	5.9	460	23.1
Range:			(63–81)	(3.1–7.7)	(190–800)	(8.2–42.0)
Control	10	163	73	6.8	58.2	5.3
Range:			(63–89)	(5.1–9.2)	(42.0–75.0)	(1.5–10.5)

TABLE III

Rate of disappearance of injected cholate from plasma of nephrotic rats

Type of rat	No. of rats	Average weight *gm.*	Plasma cholate concentration, *mg./100 cc.* Before injection	After injection* Immediately	One hour	Two hours
Nephrotic	11	208	11.0	56.6	19.0	16.4
Range:		(138–260)	(7.4–20.0)	(40.0–70.0)	(10.0–31.0)	(8.5–23.7)
S.E. Mean:			±1.6	±2.6	±1.9	±1.8
Control	10	249	5.7	58.0	8.4	5.6
Range:		(235–267)	(4.4–6.2)	(47.0–79.0)	(4.5–14.0)	(3.5–7.8)
S.E. Mean:			±0.17	±3.4	±0.56	±0.35

* 25 mg. sodium cholate injected intravenously.

cholate feedings fell from 417 mg. per 100 cc. to 211 mg. per 100 cc. The cholate feedings also induced an elevation of plasma cholesterol in the normal animals, their average concentration rising from 73 mg. per 100 cc. to 99 mg. per 100 cc.

DISCUSSION

The studies described above have shown that an excess of cholate is present in the blood plasma of nephrotic human subjects, as well as in the plasma of rats with experimentally induced nephrosis, whenever hypercholesteremia also is present. This excess of cholate may well be responsible for the reduced surface tension of nephrotic blood, a phenomenon first described by Clausen (11) and later confirmed by Leiter (12).

TABLE IV

Effect of cholate administration upon plasma cholesterol of nephrotic rats

Type of rat	No. of rats	Average weight *gm.*	Plasma cholesterol *mg./100 cc* Beginning	After 5 days
I. Normal				
A. Without cholate	8	110	68	64
Range:			(54–72)	(50–70)
S.E. Mean:			±1.94	±2.25
B. With cholate*	11	97	73	99
Range:			(57–92)	(61–142)
S.E. Mean:			±1.98	±8.83
II. Nephrotic				
A. Without cholate	6	124	417	211
Range:			(234–800)	(146–260)
S.E. Mean:			±80.5	±16.6
B. With cholate*	11	140	495	508
Range:			(266–678)	(214–661)
S.E. Mean:			±40.10	±68.90

* 100 mg. sodium cholate fed daily.

Other studies from this laboratory (4) have shown that hypercholatemia is almost invariably present in various clinical states of hypercholesteremia. This fact in itself might be only of incidental interest were it not for the observation (3) that when hypercholatemia is induced experimentally in the rat, a state of hypercholesteremia immediately develops. In view of these findings, the possible relationship between the excess cholate and cholesterol in the blood of the nephrotic animal may have considerable importance. This is discussed more extensively in a forthcoming study (13).

SUMMARY

An excess accumulation of bile acid (hypercholatemia) was found in the blood of human subjects with nephrosis and in the blood of rats in which experimental nephrosis had been induced by injection of anti-rat kidney serum, whenever hypercholesteremia also was present. The nephrotic rat also was found to exhibit a diminished ability to rid his blood of excess injected cholate. Finally, the feeding of cholate to nephrotic rats appeared to prevent the decrease of plasma cholesterol, which usually occurred, following its initial maximal rise.

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THE EFFECTS OF INTRAVENOUS PRISCOLINE ON CEREBRAL CIRCULATION AND METABOLISM[1]

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(Submitted for publication September 9, 1952; accepted November 14, 1952)

Priscoline hydrochloride (2-benzyl-imidazoline-hydrochloride) is a drug of many pharmacologic actions. The effects of the drug in man apparently depend upon the dosage and method of administration. It is reported to produce peripheral vasodilatation by: *1*) adrenergic blockade, inhibiting the effects of circulating adrenalin on smooth muscle cells of blood vessels; *2*) sympathetic blockade at the terminations of the sympathetic nerves in the blood vessels; *3*) direct action on peripheral vessels; and *4*) epinephrine-reversal. There may be sufficient increase in cardiac output to elevate arterial pressure even in the presence of generalized peripheral vasodilatation (1–7). The drug also has other actions not specifically related to the cardiovascular system. Clinically, priscoline has been used widely in the treatment of occlusive and spastic peripheral vascular disease because of its ability to produce an increased cutaneous and muscle blood flow (8–11). Several enthusiastic reports have appeared on the beneficial effects of priscoline on cerebral vessels in the treatment of cerebral vascular disease (12–14), and D. Engel has observed dilatation of pial vessels by capillary microscopy through a window in the skull of cats following intravenous priscoline (15). The present study was undertaken in an effort to measure the effects of large doses of intravenous priscoline on cerebral blood flow, metabolism, and other cerebral metabolic functions in man.

METHOD

Twenty-four patients, chosen from the hospital wards, were studied. Most were essentially normal, middle-aged or elderly individuals and the others had a variety of disease states. The mean age of the group was 48 years, but only 5 were under the age of 40, whereas 9 were 55 years or over. None of the subjects had acute cerebral vascular accidents. A control blood flow was performed using the modification of the original nitrous oxide technique previously described (16). The priscoline was then administered intravenously in doses of 50 to 100 mgm. in 200 ml. of normal saline over a period of 15 to 20 minutes. Thirteen patients received 100 mgm. of priscoline, 2 received 50 mgm., and 9 received 75 mgm. The second blood flow measurement was begun five to eight minutes after the intravenous solution had been started, during the period of maximal drug action. All patients developed facial flushing, conjunctival suffusion, moderate pupillary constriction, nasal stuffiness, pilomotor erection, and tachycardia. Blood pressure responses were variable. Arterial pressure measurements were made by the auscultatory method every two minutes during the procedure, with the arm held at heart level. Mean pressures were calculated from the formula

$$MP = \text{diastolic pressure} + \tfrac{1}{3} \text{ pulse pressure},$$

and agreed with mean pressures measured directly by a mercury U tube manometer in most patients. Blood samples for oxygen determinations were drawn before and after each blood flow measurement, and the average was used as the final value for arterial-cerebral venous oxygen difference and arterial oxygen saturation. The oxygen measurements were done by the spectrophotometric method of Hickam and Frayser (17). Arterial and venous oxygen tensions were estimated from the pH and the per cent oxyhemoglobin saturation, using the dissociation curves of Dill. (The values for oxygen tension when oxyhemoglobin saturation was above 90 per cent were obtained from a chart of interpolated values, using the figures of Dill, since it was impossible to read the curves accurately above this point.) Carbon dioxide content of whole blood was measured by the method described by Peters and Van Slyke (18). Carbon dioxide tension was estimated from the nomograms of Peters and Van Slyke (18). Whole blood pH was measured with the glass electrode of a Cambridge Model R pH meter at 33° C., and corrected to 38° C. by Rosenthal's factor. Samples for blood glucose were collected similarly to the oxygen samples, but the average arterial-cerebral venous glucose difference represents four arterial and four venous glucose determinations. Blood

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TABLE I—*Effects of intravenous priscoline on cerebral metabolic functions*

Pt.	Age	Diagnosis and dose	Cerebral blood flow		Arterial-cerebral venous O_2 diff.		Cerebral O_2 consumption		Arterial O_2 saturation		Arterial O_2 tension		Cerebral venous O_2 tension		Arterial CO_2 content		Arterial CO_2 tension		Arterial pH		Venous pH		Arterial-cerebral venous glucose diff.		Cerebral glucose consumption		Mean arterial pressure		Cerebral vascular resistance	
			ml./min./ 100 gm. brain		*Vol. %*		*ml. O_2/min./ 100 gm. brain*		*%*		*mm. Hg*		*mm. Hg*		*Vol. %*		*mm. Hg*						*mgm. %*		*mgm. glucose/ min./100 gm. brain*		*mm. Hg*		*mm. Hg/ml. blood/min./ 100 gm. brain*	
			$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$	$\bar{a}$	$\bar{p}$
RP	19	Inf. Hepatitis—100 mgm.	47	41	7.3	8.0	3.4	3.3	94	98	75	110	32	31	47.5	47.9	41	36	7.39	7.45	7.36	7.39	7.3	7.6	3.4	3.1	82	85	1.74	2.07
CK	55	Pul. Emphysema—100 mgm.	54	64	7.3	7.2	4.0	4.6	90	86	62	53	30	30	49.1	49.6	43	40	7.39	7.42	7.34	7.33	8.5	13.0	4.9	8.3	74	54	1.37	0.84
NC	30	Normal—100 mgm.	94	—	5.0	6.4	4.7	—	97	97	102	97	43	36	45.1	41.2	44	36	7.35	7.42	7.31	7.37	8.3	12.5	7.8	—	81	84	0.86	—
DC	64	Normal—100 mgm.	79	39	4.7	5.2	3.7	2.0	91	92	69	74	38	41	52.3	51.0	49	50	7.34	7.32	7.31	7.21	7.0	9.3	5.5	3.6	80	70	1.01	1.80
FB	53	Normal—100 mgm.	45	37	5.2	5.0	2.3	1.8	95	93	—	—	35	36	49.7	48.3	—	—	—	—	—	—	7.0	9.0	3.2	3.3	83	87	1.84	2.35
WK	61	Normal—100 mgm.	36	39	4.7	5.3	1.7	2.1	91	90	67	61	38	33	51.6	50.4	46	40	7.37	7.42	7.33	7.37	5.4	5.6	2.0	2.2	93	91	2.58	2.33
ER	67	Normal—100 mgm.	100	77	5.8	7.2	5.8	5.5	90	93	63	73	33	31	44.8	42.8	39	37	7.38	7.38	7.34	7.32	8.9	10.0	8.9	7.7	93	95	0.93	1.23
EM	43	Normal—100 mgm.	78	77	5.7	6.2	4.4	4.8	98	98	111	106	36	31	47.8	46.1	43	37	7.36	7.42	7.33	7.37	—	—	—	—	94	88	1.21	1.14
WP	46	Normal—100 mgm.	61	52	6.4	7.3	3.9	3.8	96	94	97	79	43	36	47.3	45.4	49	43	7.30	7.35	7.27	7.29	—	—	—	—	91	93	1.49	1.79
MB	54	Normal—75 mgm.	55	53	6.2	6.0	3.4	3.2	95	96	81	90	33	34	51.0	50.0	43	44	7.39	7.37	7.36	7.35	—	—	—	—	84	94	1.53	1.72
HH	40	Amyotrophic lateral sclerosis—75 mgm.	43	51	8.6	9.0	3.7	4.6	97	94	101	76	32	28	44.9	44.2	40	39	7.38	7.38	7.31	7.32	—	—	—	—	96	93	2.23	1.82
EN	36	Cerebral Vasc. Dis.—75 mgm.	54	—	4.8	3.6	2.6	—	93	93	71	73	35	46	47.6	45.5	40	41	7.40	7.37	7.38	7.35	—	—	—	—	96	100	1.78	—
GV	58	Normal—75 mgm.	54	42	5.8	—	3.1	—	—	—	—	—	—	—	—	—	—	—	7.38	7.45	7.34	7.42	—	—	—	—	117	99	2.17	2.36
JD	54	Cirrhosis—75 mgm.	67	48	6.0	6.1	4.0	2.9	92	92	68	63	32	29	—	—	—	—	7.39	7.46	7.35	7.40	—	—	—	—	88	77	1.31	1.60
CO	41	Rh. Arthritis—100 mgm.	77	—	7.6	8.2	5.9	—	96	96	—	—	30	30	—	—	—	—	—	—	—	—	—	—	—	—	87	83	1.13	—
CM	41	Normal—100 mgm.	73	—	5.7	6.8	4.2	—	99	98	—	—	34	30	—	—	—	—	—	—	—	—	—	—	—	—	94	91	1.29	—
MR	36	Emphysema—75 mgm.	49	—	7.7	8.1	3.8	—	92	94	—	—	32	32	—	—	—	—	—	—	—	—	—	—	—	—	84	86	1.72	—
LA	38	Normal—100 mgm.	57	—	4.9	4.2	2.8	—	97	97	—	—	40	44	—	—	—	—	—	—	—	—	—	—	—	—	97	93	1.70	—
EH	41	Normal—100 mgm.	40	—	6.5	6.4	2.6	—	91	89	—	—	30	30	—	—	—	—	—	—	—	—	—	—	—	—	110	92	2.75	—
IW	62	Normal—75 mgm.	33	—	7.3	8.5	3.1	—	99	99	—	—	33	30	—	—	—	—	—	—	—	—	—	—	—	—	101	85	3.06	—
RM	43	Cirrhosis—75 mgm.	46	—	4.6	4.6	2.1	—	86	87	—	—	30	31	—	—	—	—	—	—	—	—	—	—	—	—	98	95	2.13	—
FH	58	Dx. Unknown—75 mgm.	44	—	6.3	7.0	2.8	—	98	97	—	—	34	32	—	—	—	—	—	—	—	—	—	—	—	—	100	97	2.28	—
GH	55	Normal—50 mgm.	57	60	7.4	7.0	4.2	4.2	99	97	—	—	31	31	—	—	—	—	—	—	—	—	—	—	—	—	110	102	1.93	1.70
JF	62	Cirrhosis—50 mgm.	50	47	5.1	5.4	2.6	2.5	96	94	—	—	33	29	—	—	—	—	—	—	—	—	—	—	—	—	97	81	1.94	1.72
		MEAN VALUES	59	52	6.12	6.47	3.62	3.50	94.4	94.1	80.6	79.6	34.2	33.1	48.2	46.9	43.4	40.3	7.37	7.40	7.33	7.35	7.8	9.6	4.7	4.7	93	88	1.66	1.75
		p values	$0.05 < p < 0.1$		$p = 0.02$		$p > 0.4$		$p > 0.5$		$p > 0.5$		$p > 0.5$		$p < 0.01$		$p < 0.01$		$p < 0.01$		$p > 0.3$		$p < 0.02$		$p > 0.5$		$p < 0.01$		$p > 0.5$	

$$t = \frac{\text{Mean difference}}{\text{Std. error of mean diff.}}$$

$\bar{a}$ = Before priscoline.
$\bar{p}$ = After priscoline.

glucose was measured by the sodium thiosulfate titration method of Somogyi (19).

Cerebral oxygen consumption (CMR O_2)
= cerebral blood flow (CBF) × arterial − cerebral venous oxygen difference (A − V O_2)

Cerebral glucose consumption (CMR Gl.)
= CBF × arterial − cerebral venous glucose difference (A − V Gl.)

Cerebral vascular resistance (CVR)
$$= \frac{\text{mean arterial pressure (MAP)}}{\text{CBF}}$$

RESULTS

The data obtained in this study are presented in detail in Table I. There is an apparent reduction in CBF from a mean of 59 to 52 ml./min./100 gm. brain. Though strictly speaking this is not a statistically significant change, it is felt that it represents a definite trend for reduction of CBF following priscoline. This feeling is strengthened by the statistically significant increase in A–V O_2 from 6.1 to 6.5 vol. %, with no alteration in CMR O_2. Though glucose observations were made on only 7 patients, there is a statistically significant increase in A–V Gl. from a mean of 7.8 to 9.6 mgm. %, with no change in CMR Gl.

In almost all subjects the administration of priscoline was followed by a mild increase in respiratory rate and depth. The fall in arterial CO_2 content (48.2 to 46.9 vol. %), the increase in arterial pH (7.37 to 7.40), and resultant drop in arterial CO_2 tension (pCO_2) (43.4 to 40.3 mm. Hg), all of which are statistically significant changes, seem to confirm the visual observation that the patients were over-ventilating. There was no significant change in cerebral venous pH, arterial O_2 saturation, arterial or cerebral venous O_2 tension, or cerebral vascular resistance. There was a small but significant drop in arterial pressure from a mean of 93 to 88 mm. Hg.

It is of interest that some of the earliest observable clinical effects of priscoline in these patients, aside from tachycardia, were the conjunctival suffusion, nasal congestion, mild pupillary constriction, facial flushing, and increased facial heat and dryness, which closely resembled the effects of a bilateral stellate ganglion block. The known effect of priscoline in producing sympathetic blockade was probably responsible for this phenomenon. The intense pilomotor reaction of priscoline usually occurred later, and the resultant sensation of chilliness did not appear until the measurements on cerebral metabolism had been completed. All patients suffered mild to severe postural hypotension which persisted for at least 30 minutes after administration of the drug was completed.

DISCUSSION

The data indicate that priscoline apparently has an effect on cerebral vessels that differs from that on the vessels of skin and muscle. Unfortunately the data do not permit a final interpretation of the manner in which priscoline seems to produce a slightly diminished CBF and widened A–V O_2. It is possible that administration of the drug induced moderate hyperventilation, with reduction of arterial pCO_2, and subsequent decrease of CBF, since hyperventilation is known to diminish CBF (20), although the absence of evidence of active vasoconstriction and the relatively small reduction in arterial pCO_2 make this concept doubtful. Priscoline may produce a chain of events similar to that postulated for aminophylline (21); it may produce mild cerebral vasoconstriction causing a decrease in CBF, with mild anoxia of cerebral tissue (decreased O_2 content of cerebral venous blood), and resultant increase of cerebral CO_2 tension. This may in turn produce hyperventilation, with resultant diminution in arterial pCO_2. The complete lack of correlation between changes in arterial pressure and CBF indicates that the apparent reduction in CBF is not due to the fall in arterial pressure, as seems to be the case with histamine (22).

Though the effects of priscoline on cerebral metabolic functions are somewhat variable, there is no evidence that priscoline produces cerebral vasodilatation. The ineffectiveness of the sympathetic blocking effect of priscoline on cerebral circulation could have been anticipated by the known lack of response of CBF to stellate ganglion block (23, 24).

The apparent clinical effects of priscoline which have been attributed to its action on cerebral circulation or metabolism (13–15) are difficult to explain on the basis of the results obtained in this study. It would seem that such doses of prisco-

line are actually prejudicial to CBF and that the drug should be used with caution in cerebral vascular disease. Although in none of our subjects was there reason to suspect acute cerebral vascular changes which might have been reversed by a vasodilating agent, most of these subjects were in the age group in which "subclinical" cerebral vascular disease is almost uniformly found (25). Final conclusions concerning the possible efficacy of priscoline in the treatment of cerebral vascular disease must depend upon carefully controlled clinical observations.

SUMMARY

1. Intravenous priscoline, in doses varying from 50 to 100 mgm., administered in 15 to 20 minutes, results in an apparent, though not statistically significant, decrease in CBF, an increase in A–V O_2 and A–V Gl., a slight decrease in mean arterial pressure, a decrease in arterial CO_2 content and arterial pCO_2, a rise in arterial pH, with no alteration in CMR O_2, CMR Gl., CVR, arterial O_2 saturation, arterial pO_2, and cerebral venous pO_2.

2. It is postulated that priscoline produces mild cerebral vasoconstriction and slight hypoxia of brain tissue, though its effects are variable.

3. This study does not offer a satisfactory explanation for the reported beneficial clinical effects of priscoline in the treatment of cerebral vascular disease.

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STUDIES OF PULMONARY HYPERTENSION I. PULMONARY CIRCULATORY DYNAMICS IN PATIENTS WITH PULMONARY EMPHYSEMA AT REST[1]

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Studies of the pulmonary circulation in patients with pulmonary emphysema have been reported from several laboratories (1–11). These reports include observations at rest, during exercise, after intravenous digoxin, and during and after breathing low and high oxygen concentrations in the inspired air. Pulmonary hypertension is frequently observed at rest in these patients during cardiac catheterization. In most instances pulmonary artery pressure increases even after mild exercise, whereas the pulmonary artery pressure of normal subjects remains unchanged. Induced anoxia (breathing 10 per cent or 13 per cent oxygen in nitrogen) causes a definite rise in the pulmonary artery pressure, while breathing 100 per cent oxygen reduces the pressure slightly. Furthermore, there is a significant negative correlation between the magnitude of pulmonary hypertension and the oxygen saturation of the arterial blood.

The literature regarding "pulmonary capillary" pressure (12, 13) and pulmonary arteriolar resistance in patients with pulmonary emphysema is meager (8, 10, 11). The effects of hypercapnia upon the pulmonary circulation are not yet well defined (3, 4).

It is the purpose of this paper, (*a*) to report the "pulmonary capillary" pressure and pulmonary arteriolar resistance of 18 patients with pulmonary emphysema at rest and (*b*) to correlate various determinants including the partial pressure of CO_2 in the arterial blood (P_{aco_2}) with pressure and resistance in the pulmonary circuit. In a subsequent manuscript (14) the hemodynamic effects of induced hypercapnia (breathing either 3.6 per cent or 6 per cent CO_2 in the inspired air) will be described in normal subjects and in patients with chronic pulmonary disease.

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MATERIAL AND METHOD

Eighteen patients were studied of whom 16 were men and 2 women, ranging in age from 38 to 76 years. All showed clinical, radiological and laboratory evidence of pulmonary emphysema. Two of the patients (D. C. and C. N.) had pulmonary fibrosis and one (C. N.) also had hypertensive cardiovascular disease. In three patients (C. N., J. M., and J. G.), the electrocardiogram showed evidence of right ventricular hypertrophy. Three patients (J. K., J. G., and M. M.) were digitalized for congestive heart failure prior to cardiac catheterization. In all cases the ratio between the residual volume and total capacity of the lung (RV/TC) was 48 per cent or more.

Lung volume and its subdivisions were determined by the helium dilution method described by Meneely and Kaltreider (15). The blood oxygen content and capacity were determined according to the method of Van Slyke and Neill (16). The technique for measuring partial pressure of CO_2 and O_2 in arterial blood (P_{aco_2} and P_{ao_2}) was adapted from the method of Riley, Proemmel and Franke (17).

Cardiac catheterization was carried out in each patient 2 or 3 hours after a light breakfast, according to the method of Cournand and Ranges (18). A single or double lumen catheter was employed. The catheter was first advanced as far as possible into the distal radicle of one of the pulmonary arteries in order to occlude its lumen. The "pulmonary capillary" pressure was recorded by the method of Hellems, Haynes and Dexter (12, 13), satisfying the criteria of Fowler and his associates (19). With the double lumen catheter, the "pulmonary capillary" pressure and the pulmonary artery pressure were recorded simultaneously. When a single lumen catheter was used, the "pulmonary capillary" pressure was measured first and pressure was recorded continuously as the catheter was slowly withdrawn until its tip was just distal to the bifurcation of the pulmonary artery.

TABLE I—*Summary data of measurements of pulmonary function and circulation in 18 patients with pulmonary emphysema*

Case	Age	Sex	Vital capacity Obs.	Vital capacity Pred.	Residual volume Obs.	Residual volume Pred.	$\frac{RV}{TC} \times 100$	Arterial blood Sat.	Arterial blood P_{aCO_2}	Arterial blood P_{aO_2}	O_2 cons. *cc./min./M²BSA*	Cardiac output *L./min.*	Cardiac index *L./min./M²BSA*	Pulmonary artery pressure *mm. Hg* S	D	M	"Pulmonary capillary" pressure *mm. Hg*	PAm–PCm gradient *mm. Hg*	Total pulmonary resistance *dynes sec.-cm.⁻⁵*	Pulmonary arteriolar resistance *dynes sec.-cm.⁻⁵*	Right auricular mean pressure *mm. Hg*
			cc.	%	*cc.*	%	%	%	*mm. Hg*												
E. S.	38	F	2370	75	2220	247	48	93	—	72	169	9.6	6.1	17	7	9	6	3	70	25	2
L. F.	54	M	2020	61	2540	171	56	98	36	71	168	4.2	3.2	15	9	13	8	5	237	95	5
D. C.	72	M	1660	43	2010	110	56	85	42	64	126	7.6	3.6	23	9	13	10	3	131	32	9
E. J.	49	M	2410	61	1950	160	45	92	49	73	156*	7.4	4.0	25	9	15	9	6	162	65	2
L. P.	66	M	2180	66	2020	135	48	80	42	45	152	8.6	4.9	25	12	16	10	6	149	57	6
T. J.	45	M	2170	59	3900	351	64	94	40	59	136	6.5	3.9	25	10	16	7	9	197	99	3
R. M.	67	M	2600	72	3960	243	60	92	34	63	138	4.7	2.6	28	12	17	9	8	284	132	2
H. C.	63	M	1680	45	1820	110	52	93	42	65	152	4.5	2.5	28	14	20	7	13	358	232	8
H. W.	52	M	1820	50	3090	186	63	84	35	52	162	7.0	4.3	29	10	21	3	18	240	206	2
B. R.	51	M	1690	47	2280	136	58	83	43	50	160	5.8	3.4	38	15	22	10	12	303	166	6
J. T.	62	M	914	27	1530	336	85	81	57	65	131	3.5	2.3	37	16	24	6	18	554	408	6
C. N.	55	M	1340	39	1510	98	53	90	55	63	108	3.4	2.3	34	11	26	−1	27	610	638	0
J. M.	52	M	1370	35	5400	335	80	93	45	60	164	5.5	3.8	31	20	27	7	20	410	292	5
J. K.	76	M	990	32	3680	261	79	89	60	62	166	3.8	2.7	46	19	27	12	15	558	314	7
J. C.	65	M	920	28	3890	265	81	91	48	70	196	10.7	6.6	46	21	28	9	19	412	280	6
F. Z.	48	M	2000	53	2890	258	59	90	45	60	125	4.5	2.9	38	21	30	9	21	532	370	4
J. G.	61	M	920	28	3430	235	79	82	71	48	145	4.0	3.2	37	20	31	9	22	780	440	7
M. M.	63	F	890	37	3000	277	77	74	67	46	155	4.7	3.3	75	28	49	10	39	834	666	7

* Oxygen consumption obtained at rest the following day.

A No. 19 needle was inserted into either the brachial or femoral artery. The cardiac output was determined according to the direct Fick principle. Oxygen consumption was measured by a method similar to that described previously (*20*). Mixed venous and arterial blood samples were obtained simultaneously as the oxygen consumption was determined. Pressures were recorded in both the pulmonary and systemic arteries after measuring the cardiac output. Finally, the catheter was withdrawn to the right ventricle, right auricle, and superior vena cava. Blood samples and pressure records were obtained from each site.

Pressures were recorded by means of a Statham strain gauge connected to an amplifier in a multi-channel direct-writing oscillograph.[2] The electrocardiogram and pneumogram were recorded simultaneously. The pressure records were calibrated with a mercury manometer. Systolic and diastolic pressures were measured for at least three respiratory cycles and the average values calculated. Mean pressures were measured by planimetric integration of the pressure tracings during at least two respiratory cycles. The arbitrary zero point of all pressures was 6.5 cm. below the angle of Louis with the patient in a recumbent position.

Total pulmonary resistance and pulmonary arteriolar resistance were calculated as follows (*21*):

$$TPR = \frac{PAm}{\dot{Q}_b} \times 1332$$

$$PAR = \frac{PAm - \text{“PCm”}}{\dot{Q}_b} \times 1332$$

where TPR and PAR = resistance in dynes-sec.-cm.$^{-5}$

" PAm = mean pulmonary artery pressure in mm. Hg.

" "PC" = mean "pulmonary capillary" pressure in mm. Hg.

" $\dot{Q}_b$ = cardiac output in cc./second.

" 1332 = conversion factor from mm. Hg to dynes/cm.[2]

OBSERVATIONS

The pertinent data are summarized in Table I. Some of the data have been subjected to statistical analysis. Correlations between the various determinants and the pulmonary pressures and resistances are shown in Table II and plotted in Figures 1 and 2.

1. *Pulmonary artery and "capillary" pressures and gradient*

The mean pulmonary artery pressure exceeded 15 mm. Hg in 14 of the 18 patients. The highest

[2] Sanborn Poly-Viso Cardiette

TABLE II

Coefficients of correlation between various determinants in 18 patients with pulmonary emphysema

	Mean pulmonary artery pressure	Mean "pulmonary capillary" pressure	Total pulmonary resistance	Pulmonary arteriolar resistance
Arterial blood p_{CO_2} (P_{aCO_2})	+0.693**	+0.284	+0.742**	+0.651**
$\frac{RV}{TC} \times 100$	+0.624**	+0.081	+0.544*	+0.420
Arterial blood O_2 saturation	−0.490*	−0.002	−0.318	−0.312
Cardiac index	−0.396	+0.208	−0.518*	−0.452
Arterial blood p_{O_2} (P_{aO_2})	−0.426	+0.093	−0.085	−0.272
Right auricular pressure	+0.292	+0.643**	+0.279	−0.114

** Highly significant.
* Significant.

pressure was 49 mm. Hg. The RV/TC of this patient was 77 per cent, the arterial blood oxygen saturation 74 per cent, and the P_{aCO_2} 67 mm. Hg. In general, the degree of pulmonary hypertension varied directly with the severity of hypercapnia, emphysema and anoxia, and inversely with the vital capacity. The mean pulmonary artery pressure did not correlate significantly with either cardiac index or "pulmonary capillary" pressure.

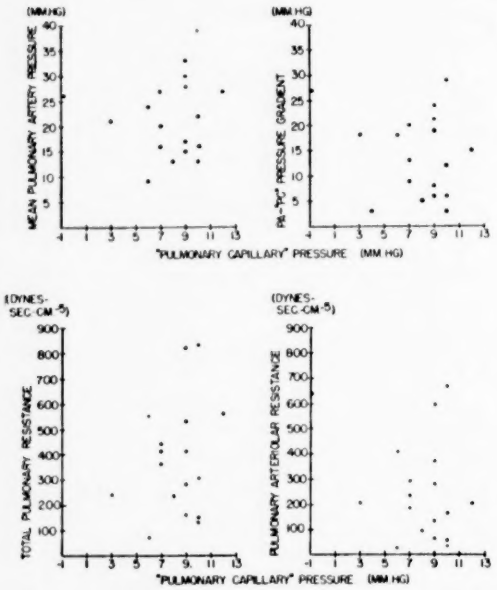
MEAN PULMONARY ARTERY PRESSURE (MM HG)
PA-"PC" PRESSURE GRADIENT (MM HG)
"PULMONARY CAPILLARY" PRESSURE (MM HG)
TOTAL PULMONARY RESISTANCE (DYNES-SEC-CM-5)
PULMONARY ARTERIOLAR RESISTANCE (DYNES-SEC-CM-5)
"PULMONARY CAPILLARY" PRESSURE (MM HG)

FIG. 1. "PULMONARY CAPILLARY" PRESSURE IN PULMONARY EMPHYSEMA IS PLOTTED AGAINST PULMONARY ARTERY PRESSURE, THE PA—"PC" GRADIENT, TOTAL PULMONARY RESISTANCE AND PULMONARY ARTERIOLAR RESISTANCE

No significant relationship exists between "pulmonary capillary" pressure and any of these factors.

In none of these 18 patients did the "pulmonary capillary" pressure exceed 12 mm. Hg, the upper limit in normal subjects (*22*). A representative tracing of patient M. M. is shown in Figure 3.

The pulmonary artery-"pulmonary capillary" pressure gradient was greater than 10 mm. Hg (the upper limit of normal) in 11 of the 18 patients. The correlation between the gradient and the pulmonary artery pressure was highly significant (R = + 0.930). Similarly, the gradient

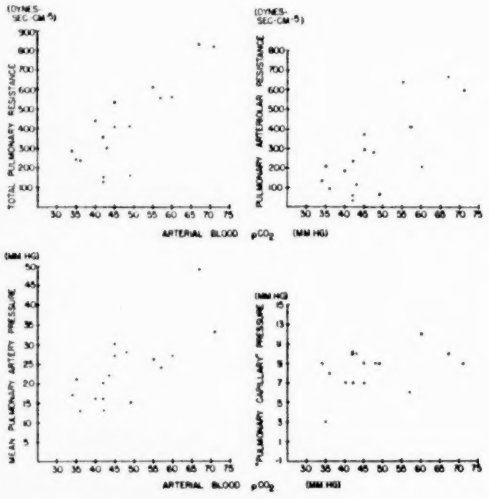
TOTAL PULMONARY RESISTANCE (DYNES-SEC-CM-5)
PULMONARY ARTERIOLAR RESISTANCE (DYNES-SEC-CM-5)
ARTERIAL BLOOD pCO_2 (MM HG)
MEAN PULMONARY ARTERY PRESSURE (MM HG)
"PULMONARY CAPILLARY" PRESSURE (MM HG)
ARTERIAL BLOOD pCO_2 (MM HG)

FIG. 2. THE P_{aCO_2} IN PULMONARY EMPHYSEMA IS RELATED TO THE PRESSURE AND RESISTANCE IN THE PULMONARY CIRCUIT

Note the significant correlation between the P_{aCO_2} and each of the following: pulmonary artery pressure, total pulmonary resistance and pulmonary arteriolar resistance. There is no correlation between the P_{aCO_2} and "pulmonary capillary" pressure. See text for discussion.

correlated significantly with the P_{aCO_2} ($R = +0.557$). No significant correlation was found between the gradient and "pulmonary capillary" pressure, arterial blood oxygen saturation, P_{aO_2} or cardiac index.

2. Pulmonary resistance

The total pulmonary resistance exceeded the upper limit of normal (250 dynes-sec.-cm.$^{-5}$) in 14 patients. Pulmonary arteriolar resistance was greater than 150 dynes-sec.-cm.$^{-5}$ (the upper limit of normal) in 13 patients. Pulmonary resistance was increased in all patients whose mean pulmonary artery pressure was 20 mm. Hg or more. Correlations of the pulmonary resistance with various determinants are presented in Table II. Neither the total pulmonary resistance nor the pulmonary arteriolar resistance correlated significantly with "pulmonary capillary" pressure ($R = 0.020$ and -0.353, respectively).

3. Cardiac output and index

Cardiac output and index varied considerably among patients. The cardiac index of six patients was less than 2.8 liters/minute/M_2 and exceeded 5.0 liters/minute/M_2 in two patients. These indices approximate the lower and upper limits of normal subjects respectively (21). There was a significant negative correlation between cardiac index and total pulmonary resistance. Cardiac index did not correlate significantly with the mean pulmonary artery pressure, mean "capillary" pressure, or pulmonary arteriolar resistance.

4. Anoxia and hypercapnia

The arterial blood oxygen saturation of all but two patients was less than 94 per cent. The lowest value was 74 per cent. P_{aO_2} of all patients was considerably reduced. A significant negative correlation was demonstrated between the arterial blood oxygen saturation and mean pulmonary artery

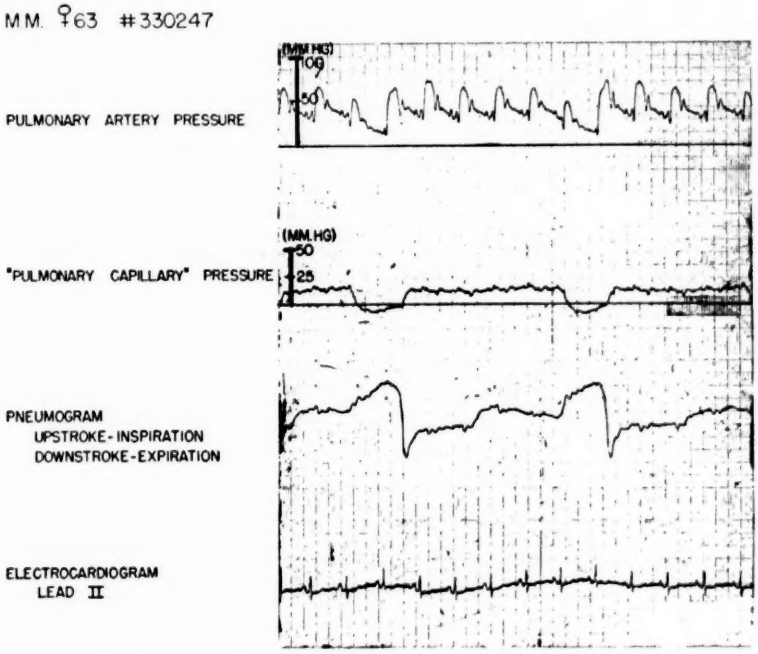

FIG. 3. TRACINGS OF PULMONARY ARTERY AND "PULMONARY CAPILLARY" PRESSURES IN PATIENT M. M.

Note the high pulmonary artery pressure with normal "pulmonary capillary" pressure and the difference in magnitude between pulmonary artery diastolic and "pulmonary capillary" pressures.

pressure. Arterial blood oxygen saturation did not correlate significantly with "pulmonary capillary" pressure, total pulmonary resistance, or pulmonary arteriolar resistance. Similarly, the $P_{a_{O_2}}$ did not correlate significantly with pressure and resistance in the pulmonary circuit.

The $P_{a_{CO_2}}$ exceeded 44 mm. Hg in 10 patients, and in all patients with a mean pulmonary artery pressure of more than 22 mm. Hg. A highly significant correlation was observed between the $P_{a_{CO_2}}$ and each of the following: mean pulmonary artery pressure, total pulmonary resistance, and pulmonary arteriolar resistance. The level of statistical significance was distinctly higher than that of the arterial blood oxygen saturation correlated with the same respective factors. On the other hand, there was no significant correlation between the $P_{a_{CO_2}}$ and either "pulmonary capillary" pressure or cardiac index.

5. Lung volume studies

The absolute amount of residual air exceeded normal in all but one patient, and the ratio between residual volume and total capacity (RV/TC) was more than 48 per cent in all cases. Conversely, the vital capacity was less than 76 per cent of the value predicted according to age and height in each instance. The RV/TC ratio correlated significantly with both mean pulmonary artery pressure and total pulmonary resistance. There was no correlation between the RV/TC ratio and either pulmonary arteriolar resistance or "pulmonary capillary" pressure.

DISCUSSION

The mechanism for the production of pulmonary hypertension in pulmonary emphysema is quite different from that in mitral stenosis and left ventricular failure. In the latter conditions, the increasing pressure is primarily registered in the left auricle and is transmitted to the pulmonary vein, "pulmonary capillary," and pulmonary artery. The pulmonary hypertension, therefore, is essentially "post-capillary" in nature. Factors such as anoxia and secondary changes of the pulmonary vascular tree may also play a role in elevating the pulmonary artery pressure (9, 23).

One important anatomic change in pulmonary emphysema is the destruction of small vessels in the lung with consequent restriction and narrowing of the vascular bed. This distorts the ratio between pulmonary blood flow and vascular capacity and is at least partly responsible for the production of chronic pulmonary hypertension. Our studies, and those of other investigators (8, 11) have shown that the pulmonary artery pressure of these patients may increase three- or fourfold when the "pulmonary capillary" pressure is still within the normal range. These observations suggest that the pulmonary hypertension produced in pulmonary emphysema is essentially "pre-capillary" in nature. Since the "pulmonary capillary" pressure is an index of left auricular pressure (24), our data support the concept that patients with pulmonary emphysema show no evidence of left ventricular failure (4, 8). The changes in the lungs and in the right side of the heart are usually progressive and eventually irreversible.

The findings of normal "pulmonary capillary" pressure in these patients were in agreement with the observations of Dexter and his associates (8) and Fowler, Westcott, and Scott (25). The latter investigators (25) observed a significant correlation between the "pulmonary capillary" pressure and the pulmonary artery diastolic pressure in 54 subjects, including 10 patients with chronic pulmonary disease, predominantly pulmonary emphysema. The data on our 18 patients with pulmonary emphysema did not yield a significant correlation between the "pulmonary capillary" pressure and the pulmonary artery diastolic pressure ($R = +0.416$). The discrepancy is explained in that pulmonary hypertension was absent or minimal in many of their patients. The pulmonary artery diastolic and "pulmonary capillary" pressures in those patients were both low, and a good correlation between the two would be expected.

Patients with mitral stenosis and left ventricular failure show a parallel elevation of both pressures, because the pulmonary artery diastolic pressure is directly related to the "pulmonary capillary" pressure. Our unpublished observations in patients with mitral stenosis show an excellent correlation between the "pulmonary capillary" pressure and the pulmonary artery diastolic pressure as long as the "pulmonary capillary" pressure remains under 30 mm. Hg. When the "pulmonary

capillary" pressure exceeds 30 mm. Hg, there may be a precipitous increase in the pulmonary artery diastolic pressure and the two pressures no longer correlate. These observations were confirmed by the recent data of Lukas and Dotter (26). This distinction between patients with pulmonary emphysema and those with mitral stenosis is important because the mechanism of pressure change in the pulmonary circuit is quite different in the two diseases.

In patients with pulmonary emphysema, the main physiologic disturbances in the ventilatory and circulatory systems are anoxia and hypercapnia. The effects of anoxia on the pulmonary circulation in man have been studied and discussed by many workers (4, 10, 11, 27, 28), and the importance of anoxia in the pathogenesis of pulmonary hypertension has been generally accepted (3, 4, 10). Our patients with chronic pulmonary disease showed a significant negative correlation between the degree of pulmonary hypertension and the oxygen saturation of the arterial blood. This correlation was especially significant when the oxygen saturation was less than 82 per cent (4). The resulting pulmonary hypertension is probably caused by pulmonary vasoconstriction with increased pulmonary arteriolar resistance (10). Fowler and his co-workers (10) have shown that breathing 13 per cent oxygen in nitrogen caused a consistent rise in mean pulmonary artery pressure and mean pulmonary arteriolar resistance above the control value but did not alter the "pulmonary capillary" pressure or cardiac output. Our observations (29) in several patients with pulmonary emphysema agree with those reported by Fowler and his associates. Other factors contributing to pulmonary hypertension may be polycythemia and hypervolemia resulting from anoxia (3, 4, 30). Polycythemia and hypervolemia may cause engorgement of the pulmonary vessels with further reduction of the distensibility of the pulmonary vascular bed. This may enhance pulmonary hypertension whenever the blood flow is increased.

Cournand and associates (3, 4) have stated, "The influence of CO_2 retention in the blood upon the pulmonary circulation is not as yet well defined." The blood CO_2 is frequently elevated in patients with moderate and marked pulmonary emphysema. Harvey and her associates (30) demonstrated a highly significant correlation between the P_{aCO_2} and mean pulmonary artery pressure in a recent study of 48 cases of chronic pulmonary disease. They found no correlation between P_{aCO_2} and cardiac index. The present studies also show a highly significant correlation between the P_{aCO_2} and the mean pulmonary artery pressure and pulmonary resistance. In contrast, no significant correlation was found between the P_{aCO_2} and either "pulmonary capillary" pressure or cardiac index. We are fully aware that correlation and causation are not the same. However, the findings suggest that hypercapnia as well as anoxia may be important in the genesis of pulmonary hypertension in patients with chronic pulmonary disease. This is supported by our studies of patients with chronic pulmonary disease, as they breathed 6 per cent CO_2. The mean pulmonary artery pressure rose during the period of CO_2 breathing. This was not due to anoxia, because there was a concomitant increase in the arterial blood oxygen saturation in some patients. The implications and significance of these changes during induced hypercapnia will be presented in a subsequent paper (14). The general cardiocirculatory effects of hypercapnia in patients with chronic pulmonary diseases have been discussed elsewhere (31–33).

SUMMARY

Hemodynamic studies are reported in 18 patients with pulmonary emphysema at rest.

1. The degree of pulmonary hypertension varied directly with the severity of emphysema, hypercapnia and anoxia.

2. "Pulmonary capillary" pressure was normal in all cases.

3. The pulmonary artery-"pulmonary capillary" pressure gradient exceeded normal in 11 patients and correlated significantly with pulmonary artery pressure and with the severity of hypercapnia.

4. Total pulmonary resistance and pulmonary arteriolar resistance exceeded normal in all instances of pulmonary hypertension but correlated well only with the degree of CO_2 retention.

5. Cardiac output and index were variable. Cardiac index correlated negatively with the total pulmonary resistance.

6. The oxygen saturation of arterial blood varied inversely with mean pulmonary artery pres-

sure but did not parallel the other determinants. The oxygen tension of arterial blood did not vary predictably with any of the determinants.

7. The carbon-dioxide tension of arterial blood correlated closely with mean pulmonary artery pressure, total pulmonary resistance and pulmonary arteriolar resistance, but not with the "pulmonary capillary" pressure.

8. The ratio of residual volume to total capacity (RV/TC) varied directly with mean pulmonary artery pressure and total pulmonary resistance.

Certain mechanism in the pathogenesis of pulmonary hypertension are discussed in detail. The role of anoxia is confirmed and attention is directed to the probable importance of carbon-dioxide retention in elevating pulmonary artery pressure and increasing pulmonary vascular resistance.

ACKNOWLEDGMENT

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SIMULTANEOUS DISTRIBUTION OF T-1824 AND I^{131}-LABELLED HUMAN SERUM ALBUMIN IN MAN [1]

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The dilution of injected dyes (1–3), especially Evans Blue (T-1824) (4), has gained wide acceptance as a measure of plasma volume (5, 6). Recently, such an interpretation of the "T-1824 space" has been questioned. The challenges are based largely upon discordant volumes of distribution obtained after the simultaneous administration of T-1824 and labelled red blood cells (7–14), or T-1824 and radioactive plasma proteins (15–18). Although others have been unable to confirm these differences (19–22), the reported divergences are in agreement, both in direction and magnitude. In every instance T-1824 was diluted to a greater extent than its companion indicator, and the ratios of T-1824 space/Indicator X space exceeded unity by 10 to 20 per cent.

The intravascular retention of T-1824 depends upon the combination of free dye anions with serum albumin (23). Two hypotheses have been advanced to explain the discrepant results obtained with T-1824 and other indicators. First, there might be early abstraction of T-1824 by phagocytosis or staining of tissues (15, 24, 25). Secondly, a disproportionate loss of T-1824 might occur during mixing by penetration into erythrocyte-poor (7–14, 25, 28) or even extravascular channels (16, 17, 25–29). The first explanation rejects T-1824 as an adequate protein label; the second explanation questions the immediate distribution of the T-1824-albumin complex.

Evidence points to the formation of a stable bond between T-1824 and albumin *in vitro* (30–33). The rapidity of this union, its stability and late metabolic fate have not been assessed *in vivo*. Much work has demonstrated the validity of I^{131} as an albumin tag (34–38) and reference standard (37, 38). Experiments were therefore designed to evaluate the vagaries of dye behavior by comparing the simultaneous transit of T-1824 and I^{131}-labelled human serum albumin in man. Attention was directed to the very early and the very late fate of T-1824 in order to elucidate those factors operative during the middle or "plasma volume" phase of dye dilution.

Transport of T-1824 was examined during the time when the concentration of unbound dye is maximal ("first circulation phase") and during the later period when the rate of removal from the blood stream is probably independent of the mixing phenomena ("late metabolic phase").

The studies indicated that union of dye and protein *in vivo* is complete and virtually instantaneous and that the disappearance of T-1824 thereafter approximates the actual exchange of albumin.

MATERIALS AND METHODS

Iodinated human serum albumin (I. H. S. A.) which incorporated two moles of iodine with one mole of albumin was obtained from a commercial source.[5] Initially, this preparation contained 200–400 μc of I^{131} and 3 to 5 mgm. of albumin in 1 ml. Upon receipt in our laboratory, the radioactivity of each batch of I.H.S.A. was reassayed. In many instances, the nitrogen content was verified by micro-Kjeldahl digestion and the homogeneity of the albumin was confirmed by electrophoresis and ultracentrifugation. Residual unbound I^{131} was measured by dialysis against distilled water for twenty-four hours and by precipitation with trichloracetic acid (TCA). Radioactivity recovered in the dialysis bath or in the TCA supernatant never exceeded 2 per cent of the total activity which was administered in any experiment.

Radioactivity was measured with a thin (1.4–1.8 mgm./cm^2) mica-end window Geiger-Mueller counter. Dupli-

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cate 0.5 ml. aliquots of plasma were pipetted onto filter paper discs cemented to the bottom of weighed planchets. One drop of alkaline 1 per cent gelatin supplemented with carrier iodide was added to each. Dried planchets were counted sufficiently long to reduce the standard deviation of the counts to less than 1 per cent. Geiger-Mueller tube characteristics and geometry were maintained constant. The self-absorption of each sample was corrected from a mass absorption curve.

Specimens for colorimetry were collected in siliconed test tubes containing 1 mgm. of dried heparin. Blood samples were centrifuged for fifteen minutes and hematocrits for forty-five minutes at 3,000 RPM. Plasma was subjected to two additional centrifugations and separations. All hemolyzed samples and those plasma specimens which still retained turbidity after three centrifugations were discarded. Optical density of T-1824 was determined at 620 mμ in a Coleman Junior spectrophotometer using a control sample of plasma similarly diluted as a blank.

Specific gravities were determined on plasma specimens by the copper sulfate method (39). In serial observations these did not change sufficiently to warrant correction of observed plasma dye concentrations.

In studies of the first circulation, T-1824 and I.H.S.A. were mixed together, prior to administration, in proportions designed to leave at least 95 per cent of the dye in an unbound state. The concentrations were based on a theoretical maximal union of 11 to 12 moles of dye with 1 mole of albumin at pH 7.4 (30, 32). The presence of dye in a free state was substantiated by marked discoloration of cellophane strips immersed in this mixture (30). Pre-mixing of the two indicators was mandatory for comparison of their early simultaneous distribution. Work in this laboratory has shown that the addition of a marked excess of T-1824 to I.H.S.A. *in vitro* can increase the vulnerability of a portion of the iodinated protein (5 to 15 per cent) to loss of radioactivity *in vivo* (40). However, since this abnormal removal does not become manifest until several circulations through sites as yet undefined, the technique of pre-mixing does not invalidate comparisons during the first circulation. T-1824 and I^{131}-labelled albumin were administered simultaneously by separate injections in studies of "the late metabolic phase."

In each experiment, a standard was prepared by diluting an identical amount of T-1824 and I.H.S.A. in distilled water to make a total volume of 1,000 ml. The distributions of T-1824 and I.H.S.A. were contrasted by graphing the concentration of dye and radioactivity in each plasma specimen as "percentage of the amount injected per ml. of plasma." The virtual volume occupied by each indicator at any given point can be derived by dividing this percentage into 100.

Subjects were medical students, laboratory personnel and convalescent hospital patients between the ages of 18 and 35. At the time of the determinations, subjects were recumbent and had fasted for at least twelve hours. Premedication consisted of 20 drops of Lugol's solution, given on the evening preceding the administration of I.H.S.A.

EXPERIMENTS

I. The First Circulation

Complete union of T-1824 and albumin requires less than 50 seconds at 37° C. *in vitro* (31). Consequently, the potentiality for loss of unbound dye by diffusion, "staining" or reticulo-endothelial activity should be at a maximum during earliest mixing within the blood stream.

The first circulation was isolated from subsequent phases of mixing by the Stewart method (41) as modified by Kinsman, Moore, and Hamilton (42, 43). Comparative fates of T-1824 and I.H.S.A. during the first circulation were quantified by administering them concurrently and by separately estimating the flow of plasma from the mean dilution of each indicator. Values were obtained from the relationship

$$f = \frac{60\,I}{ct},$$

where f is flow of plasma in ml. per minute
I is the amount injected (mgm. or counts)
c is the mean concentration of the injected substance (mgm. per ml. or counts per ml.)
and t is the time required for the first circulation (seconds).

TABLE I

Plasma flow rates during the first circulation
Simultaneous measurements with T-1824* and I^{131} labelled human serum albumin

Patient file no.	Weight	Hematocrit	Plasma flow rate (*ml./min.*) T-1824	I.H.S.A.	Ratio T-1824 / I.H.S.A.
	kg.				
		A. Central Circulation**			
1	75.0	36.6	4,488	4,662	0.963
2	72.7	46.7	4,785	4,588	1.043
3	70.0	52.0	3,085	3,101	0.995
4	69.5	50.5	4,123	4,278	0.964
5	76.8	51.4	2,258	2,275	0.993
7	71.1	45.2	3,078	3,081	0.999
8	53.2	43.3	4,397	4,293	1.024
	Mean		3,745	3,754	0.997
	Standard deviation		939	927	0.029
	Standard deviation of the mean		355	350	0.011
		B. Lower Extremity			
29	91.4	44.2	555	538	1.032
30 a.	87.3	34.6	512	507	1.010
b.			539	533	1.011

* T-1824 was mixed with I.H.S.A. prior to injection. The content of carrier albumin varied with specific activity but was never sufficient to bind more than 5% of the administered T-1824.

** Equivalent to plasma cardiac output.

The logarithm of the absolute concentration in each plasma sample of T-1824 (O.D. T-1824 at 620 mμ) and of iodoalbumin (counts per second per ml.) was plotted against time. Mean concentrations and t were determined by extrapolation of the descending slope of these values to exclude recirculation. Extrapolated values which were not based on at least three successive points falling in a straight line were discarded.

T-1824 and I.H.S.A. were compared simultaneously during their first circulation through the heart and lungs and during their initial circuit through the lower extremity. It was felt that observations of early mixing in two such widely different vascular systems would adequately assess the immediate fate of T-1824.

A. *Simultaneous transport through the heart and lungs*

Each subject was given a volume of 3–4 ml. containing 11 to 18 mgm. of Evans Blue and 1–3 mgm. of albumin labelled with 50 to 60 μc of I^{131}. Delivery was made from a calibrated syringe through a polyethylene catheter introduced into the subclavian vein. Injection required less than one second. Blood samples were collected continuously from a short plastic adapter attached to a 14-gauge retention needle within the femoral artery. The specimens were partitioned at measured two second in-

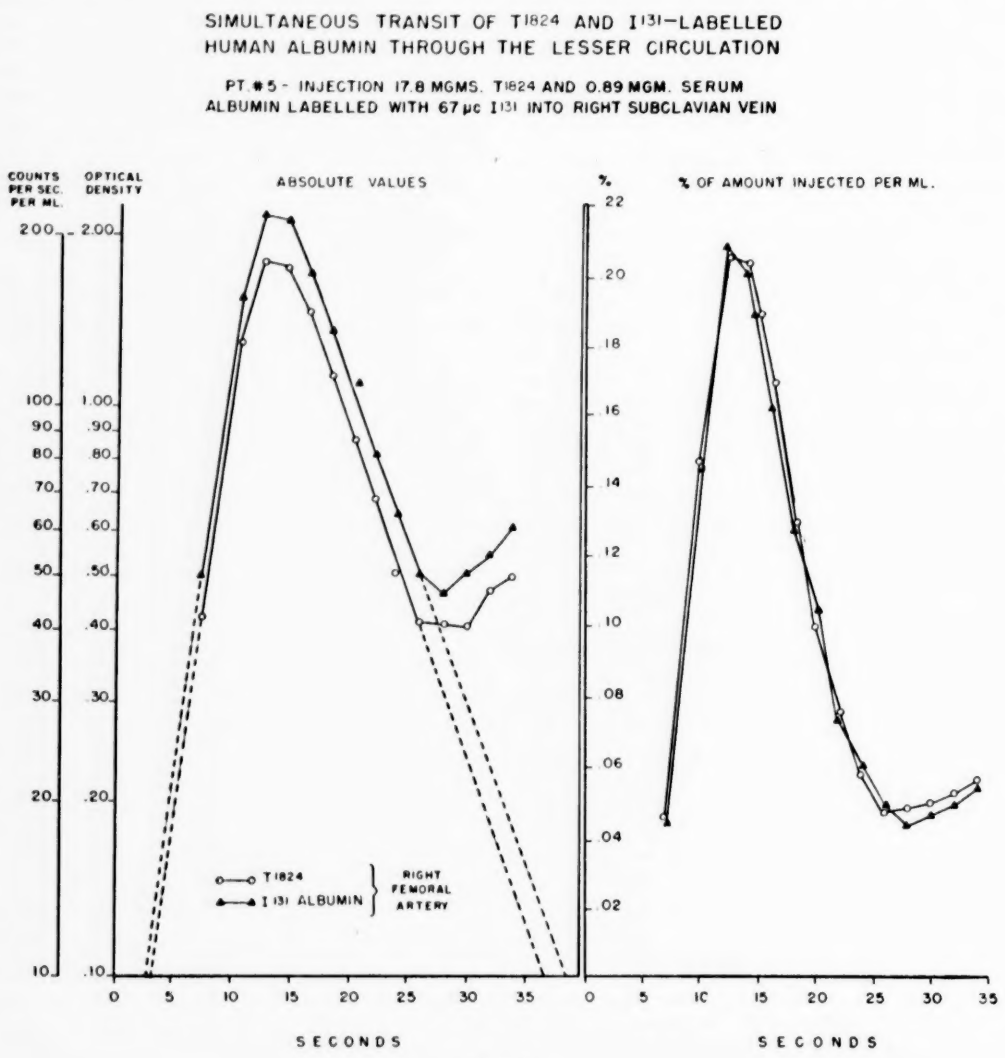

FIG. 1

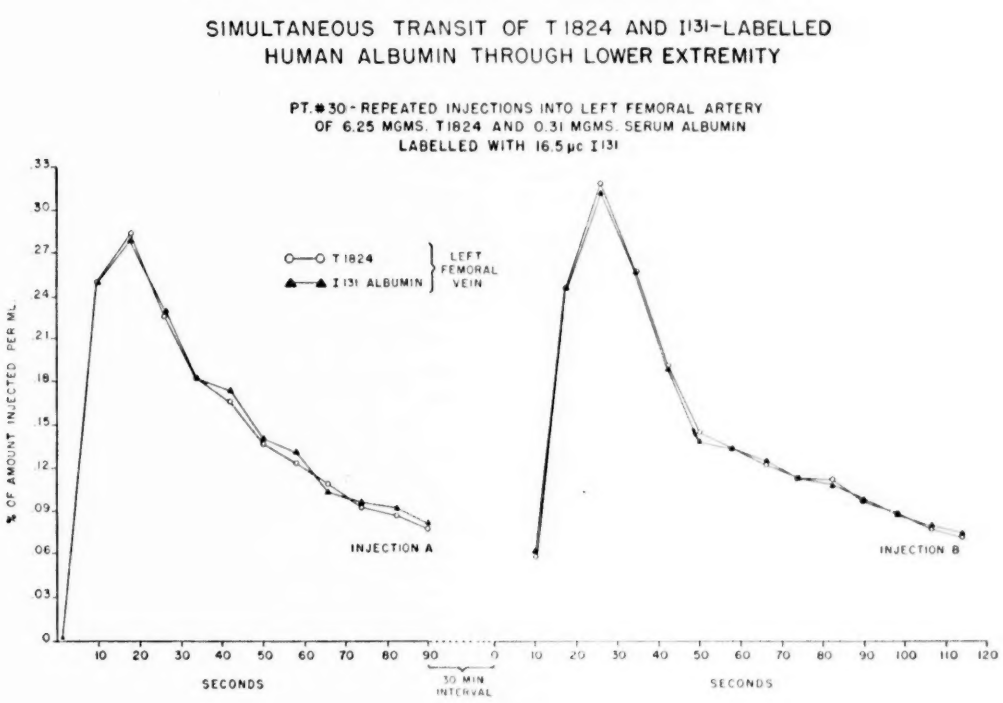
SIMULTANEOUS TRANSIT OF T 1824 AND I131-LABELLED HUMAN ALBUMIN THROUGH LOWER EXTREMITY
PT.#30 - REPEATED INJECTIONS INTO LEFT FEMORAL ARTERY OF 6.25 MGMS. T1824 AND 0.31 MGMS. SERUM ALBUMIN LABELLED WITH 16.5 μc I131
% OF AMOUNT INJECTED PER ML.
T 1824
I 131 ALBUMIN
LEFT FEMORAL VEIN
INJECTION A
INJECTION B
SECONDS
30 MIN INTERVAL
SECONDS

FIG. 2

tervals by manual direction of the adapter into test tubes. Sampling was continued well into the second recirculation.

Successful comparisons were done in seven subjects. T-1824/I.H.S.A. plasma flow ratios averaged 0.997 (Table I). Difference in minute output, stroke volume, or amount of T-1824 administered did not modify the ratio. Concentrations of the two indicators during the first circulation were superimposable when graphed as "percentage of the amount injected" (Figure 1).

Other investigators comparing the simultaneous movement of T-1824 and radioactive red blood cells also observed no preferential retention of dye within the lesser circulation (44, 45).

B. *Simultaneous transport through lower extremity*

A mixture of 6.25 to 10 mgm. T-1824 and 0.30 to 0.31 mgm. of albumin labelled with 16.5 to 20 μc I^{131} in a total volume of 2.2 to 2.5 ml. was administered from a calibrated syringe. Delivery was made in less than one second through a 23-gauge needle inserted into the femoral artery in the femoral triangle. Venous plasma specimens were obtained at eight second intervals by syringe collection from an indwelling Cournand needle in the femoral vein. Three satisfactory comparisons were performed in two individuals (Table I). Rates of "plasma flow" were derived from the mean dilutions of T-1824 and I.H.S.A. These figures may not represent the true flow values for the lower extremity because of arterial streaming, inadequate mixing and variation in circulation time through deep and superficial vasculature.

T-1824/I.H.S.A. plasma flow ratios averaged 1.018. Two determinations performed at thirty minute intervals in subject No. 30 indicate the reproducibility of the technique and the absence of a significant removal of unbound dye (24). Transport of both indicators was identical (Figure 2).

II. The Late Metabolic Phase

The rapidity of dye-protein union need not reflect the stability of the resulting complex. However, much *in vitro* evidence suggests that T-1824 and albumin combine in a tight bond. At physiological pH, there are a maximum of 11 sites in the albumin molecule for linkage with T-1824 (30, 32). Allen and Orahovats have estimated that "the binding energy from T-1824 is 8,750 calories per mole" as opposed to "an energy change of about 3,000 calories in peptide bond formation between various amino acids" (32). The complex is not disrupted by simultaneous incubation with liver slices (33) and 0.96 mole of albumin per mole of dye suffice to prevent the staining of cellophane strips immersed in 0.002 per cent T-1824 (30).

At equilibrium within the plasma, less than 0.1 per cent of the dye exists as free dye anions (32). Persist-

ence of dye within the pool of plasma proteins should be expected. Keith, Rowntree, and Geraghty in 1915 described gross retention of Brilliant Vital Red within the bloodstream for several days (1). Prolonged discoloration of plasma after the administration of T-1824 was also observed by Dawson, Evans, and Whipple (3). However, we have been unable to discover any reports of quantitative measurements of T-1824 plasma levels in humans which were extended beyond one day.

Removal of dye from the circulation will be determined by metabolic processes alone only after T-1824 has equilibrated throughout its ultimate compartment of distribution. Therefore studies were instituted to evaluate the late disappearance of the T-1824-albumin complex.

A. *Twenty-four hour plasma levels of T-1824*

Twenty-seven subjects were given 15 to 25 mgm. of T-1824. Specimens of blood were collected at intervals of five minutes during the first thirty minutes and another specimen was taken twenty-four hours following the administration of dye. A "zero-time" plasma level of T-1824 was derived by the semilogarithmic extrapolation of the early dye disappearance. Twenty-four hour plasma levels were expressed as a percentage of this initial concentration and as a percentage of the amount injected (Table II).

The residual T-1824 in the plasma averaged 50. 7 per cent after twenty-four hours, and was independent of the amount of dye given and the level of circulating plasma proteins. LeVeen and Fishman made identical observations in eighteen human subjects, and reported an average removal of 46.5% of T-1824 from the circulation in one day (46).

These twenty-four hour values are in good agreement with figures reported by other workers using I^{131}-labelled human serum albumin in man (17, 38, 47), and S^{35} (48), C^{14} (49, 50) and N^{15} (51) labelled preparations of plasma proteins in dogs.

B. *The late simultaneous distribution of T-1824 and I^{131}-labelled human serum albumin*

Ten subjects were given 25 to 50 mgm. of dye and 0.40 to 3.0 mgm. of I.H.S.A. labelled with 40 to 60 μc. of I^{131}. Both indicators were dissolved in equal volumes (10 ml.) and administered simultaneously into separate arm veins after the collection of a control plasma blank.

Fasting, morning blood specimens were secured at one or two day intervals for seven or eight days. Spectro-

TABLE II

Twenty-four hour plasma level of T-1824

Patient file no.	Weight	Serum albumin	24 hour concentration ×100 / Initial concentration *	24 hour concentration/ml. ×100 / Amount injected
	kg.	*gm. %*		
3	70.0	2.88	46.8	.0169
4	69.5	3.72	53.3	.0208
5	76.8	3.85	43.4	.0193
10	70.5	3.77	55.5	.0172
32	90.6	—	37.5	.0111
33	69.3	—	45.9	.0141
37	75.0	4.20	40.1	.0149
38	79.5	3.87	46.4	.0144
39	81.8	3.76	42.5	.0153
40	65.5	—	47.3	.0172
41	75.0	—	50.4	.0145
43	62.7	3.34	51.7	.0171
44	62.7	3.12	61.2	.0219
47	85.5	4.01	54.2	.0167
48	75.0	4.42	55.0	.0187
49	63.6	4.36	59.5	.0254
50	70.7	4.00	60.8	.0238
51	74.5	4.13	48.3	.0190
52	81.4	4.24	50.0	.0145
53	67.3	4.24	57.8	.0177
54	49.1	3.99	54.9	.0248
55	68.6	3.96	55.5	.0169
56	68.9	3.90	51.8	.0219
57	96.8	3.79	49.5	.0107
58	75.9	4.19	50.4	.0165
64	78.2	4.00	44.2	.0144
65	63.6	3.83	56.0	.0208
Mean			50.7	.0176
Standard deviation			6.2	.0038
Standard deviation of the mean			1.2	.0007

* The semilogarithmic plot of dye disappearance during the first thirty minutes was extrapolated to "zero time" to derive the initial concentration of T-1824.

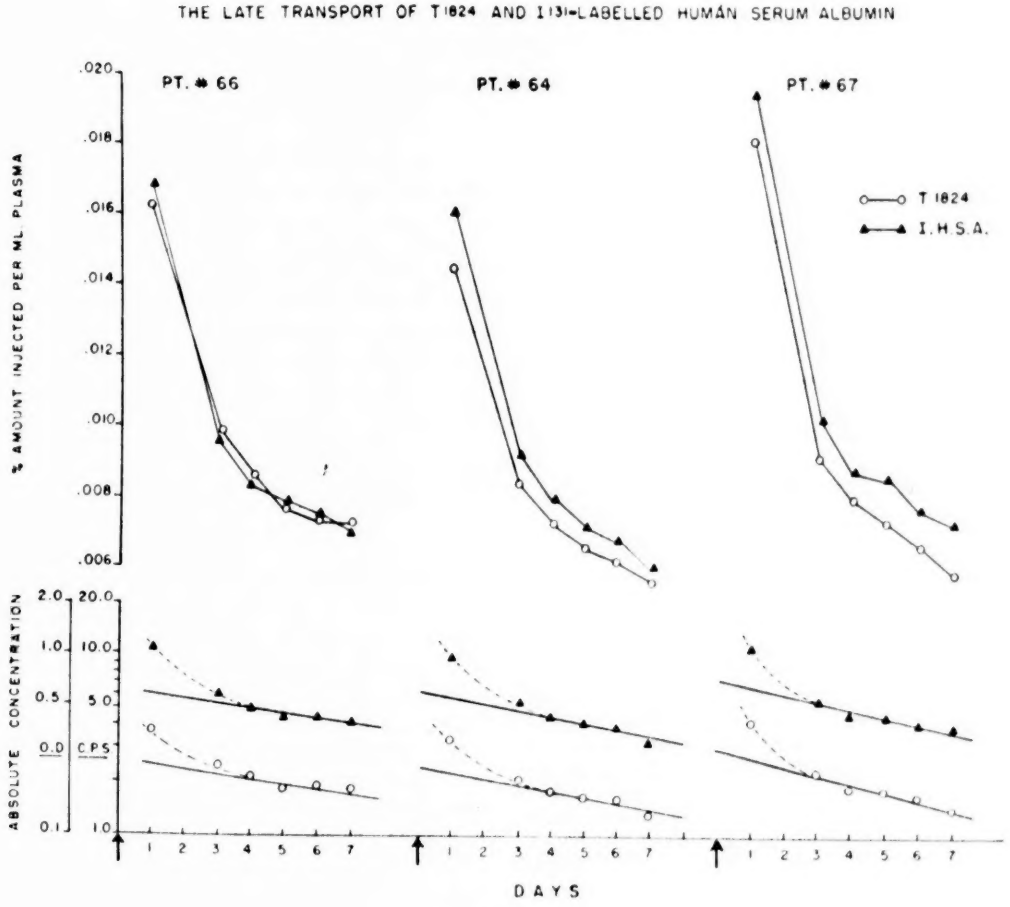

FIG. 3

photometric estimations of dye concentration were performed immediately. The initial undyed specimens of plasma were employed as colorimetric reference standards and kept frozen between analyses. The plasma content of dye in subjects who had received only 25 mgm. of T-1824 approximated 80 per cent transmittance at the end of one week. No readings exceeded this arbitrary limit of sensitivity.

The rates of disappearance of T-1824 and I.H.S.A. were compared in three ways. *1)* Daily plasma concentrations of each indicator were graphed as "percentage of the amount administered" (Figure 3). Disappearance of dye and radioactivity was approximately exponential after two to three days. *2)* "Apparent biological half-lives" were derived for T-1824 and I.H.S.A. by standard graphic analysis of their late disappearance slopes (Table III). Such analysis need not reflect true rates of turnover since observations were not extended beyond one week and since T-1824 and I.H.S.A. were not administered by infusion techniques. The measurements were intended only for semi-quantitative comparison of the two indicators. *3)* The late slopes were extrapolated to zero-time, and "total distribution compartments" were estimated for T-1824 and I.H.S.A. by dividing the amount injected by the theoretical concentration of each indicator at zero time (Table III).[6]

Three types of relationships were observed in the ten studies. In four subjects, the daily decrements of T-1824 and I.H.S.A. were superimposable (Figure 3, Pt. No. 66). In five subjects each successive sample of plasma contained progressively less T-1824 than I.H.S.A. (Figure 3,

[6] These represent minimum virtual volumes since differences in the concentrations of intravascular and extravascular protein were disregarded in the calculation. Substitution of specific activities (mgm. dye/gm. albumin or counts I.H.S.A./gm. albumin) in the denominator would not have changed the comparative relationships.

TABLE III

Comparison of the late distribution and "biological half-life" of T-1824 and I^{131} labelled human serum albumin

Patient file no.	Diagnosis	Weight	Total distribution compartment			"Biological half-life"		
			T-1824	I.H.S.A.	T-1824 / I.H.S.A.	T-1824	I.H.S.A.	T-1824 / I.H.S.A.
		kg.	*ml.*	*ml.*		*days*	*days*	
66	Post-operative: A–V fistulectomy	75.8	9,460	9,421	1.00	11.3	11.3	1.00
64	Post-operative: A–V fistulectomy	78.2	9,912	9,282	1.07	8.4	8.5	0.99
67	Post-operative: A–V fistulectomy	79.6	8,377	7,670	1.09	7.5	7.9	0.95
68	Post-operative: A–V fistulectomy	63.7	8,434	8,484	0.99	7.3	7.4	0.99
47	Laboratory worker	85.5	6,634	6,599	1.01	10.0	10.2	0.98
50	Laboratory worker	70.7	5,520	5,291	1.04	8.5	9.0	0.94
49	Laboratory worker	63.6	5,390	5,157	1.05	8.4	8.9	0.94
48	Laboratory worker	75.0	6,220	5,942	1.05	7.3	8.3	0.88
51	Laboratory worker	74.5	6,677	6,712	0.99	10.5	10.5	1.00
52	Laboratory worker	81.4	8,335	7,466	1.12	8.6	9.6	0.90
	Mean		7,496	7,202	1.04	8.8	9.2	0.96
	Standard deviation		1,614	1,535	0.04	1.4	1.2	0.04
	Standard deviation of the mean		510	485	0.01	0.4	0.4	0.01

Pt. No. 67). Only one individual demonstrated a pattern compatible with an early discontinuous abstraction of dye. In this subject, there was a divergence of the two indicators on the first day and a relatively constant difference throughout the succeeding week (Figure 3, Pt. No. 64). T-1824/I.H.S.A. "biological half-life" ratios averaged 0.96; T-1824/I.H.S.A. "total distribution compartment" ratios averaged 1.04. Values obtained with the two indicators were never more divergent than 12 per cent. All disparities were in the direction of more rapid loss and greater dilution of T-1824 and hence would suggest that these are not the result of methodological error. More studies will be necessary to define the true limits of potential divergence.

DISCUSSION

Little transcapillary exchange occurs normally within the lesser circulation (52). However, this system presents ample surface for "staining" of reticulo-endothelium or capillary endothelium since the pulmonary circulation includes "the richest capillary network in the body" (53), and lymphoid tissue within the lung exceeds that of liver or spleen (54). The peripheral circuits have less potential for phagocytosis although there is much exchange in these areas by diffusion and filtration. An identical dilution has been demonstrated for T-1824 and I^{131}-labelled albumin during their first circulation through the heart and lungs as well as the lower extremity. This indicates that the union of T-1824 and plasma protein *in vivo* is complete and prompt.

In rare instances, such as in secondary amyloidosis (55, 56) or following the administration of polyvinyl pyrrolidone (57), there may be successful competition for T-1824 by abnormally reactive substances whose dye affinities exceed those of plasma proteins.

The present studies attest to the prolonged stability of the dye-albumin complex in normal man. Over a period of one week, both T-1824 and I.H.S.A. disappeared at identical rates in four of ten simultaneous comparisons. The divergent turnovers which were observed in the remaining subjects may represent true metabolic differences.

T-1824 and I^{131} affix at different sites in the albumin molecule. The dye "tags" albumin by linkage with the epsilon ammonium of lysine (58) whereas the tyrosyl groups are labelled in the process of iodination (59). Several factors may, therefore, complicate any evaluation of the relative fates of different albumin labels. It is not known whether the albumin molecule is metabolized as a unit or by replacement of constituent peptide groups. The extent of amino acid reutilization subsequent to the breakdown of albumin within the cell is also unknown. T-1824 may be permanently removed after cellular penetration by combination of dye with the dibasic amino acids of nucleoproteins (32). In contrast, any intracellular incorporation of iodinated tyrosyl residues into newly synthesized plasma

proteins would modify the relative decrement of plasma radioactivity and prolong the apparent "half-life" of I.H.S.A. These are fundamental questions in protein chemistry and beyond the scope of this investigation. Nevertheless, the data do demonstrate that the late disappearance of T-1824 is not markedly incompatible with the actual turnover of albumin.

The concept of "total distribution compartments" has been introduced to compare the ultimate and minimum virtual volumes which T-1824 and I.H.S.A. would have occupied if their equilibration with all the exchangeable albumin had been instantaneous. Others have used similar extrapolation techniques to quantify the absolute "exchangeable pools" of slowly mixing metabolites (38, 60–62). It may be pertinent to examine the potential error which such extrapolation can introduce.

Prior to the completion of mixing, there are varying amounts of label with each aliquot of albumin in the diverse subdivisions of the true albumin "space." Thus, although a constant amount of albumin is metabolized per unit of time, there is an inconstant sampling of the labelled albumin. If albumin is catabolized in the rapidly equilibrating portion of the albumin pool, protein of high specific activity may be disproportionately lost throughout mixing. Plasma concentrations of T-1824 or I.H.S.A. may thereby be artifactually depressed at the end of mixing (*i.e.*, after two to three days) and any "space" derived by extrapolation would be too large. Similarly, if albumin metabolism occurs in areas which equilibrate slowly, protein of disproportionately low specific activity will be sampled during mixing and extrapolation to "zero time" would yield a space which is too small. The appearance of measurable amounts of dye (63) and radioactivity (64) in the bile soon after the administration of T-1824 and I.H.S.A. suggests that much of the normal breakdown of albumin occurs in areas where exchange is rapid, as in the liver.

No attempt has been made here to define that fraction of the total T-1824 "space" (commonly called "plasma volume") which is measured by the ten-minute to half-hour dilution of dye. It does not seem unreasonable that disproportionate extravascular dilution of dyed protein might occur during early mixing within the blood stream even as disproportionate metabolism of dyed protein is possible during prolonged distribution within the "total T-1824 space." Extrapolation would not account correctly for either type of disproportionate removal. The losses thereby introduced could explain the incomplete recovery of dye by vivi-perfusion techniques (25, 28) and the conflicting reports of early dye dilution (7–14, 19, 20). Obviously alternative explanations exist. However, the present evidence does not support any criticism of T-1824 methods which is based purely on delayed and unstable union of dye and protein.

SUMMARY

1. The distribution of T-1824 in normal man has been examined during the initial and the late phases of dye dilution and metabolism.

2. Dilution of T-1824 did not exceed that of concurrently administered I^{131}-labelled human serum albumin in seven measurements of the first circulation through the heart and lung and in three studies of the first circulation through the lower extremity. No support could be found for the concept of immediate abstraction of dye by tissues.

3. In twenty-seven studies, the twenty-four hour plasma levels of T-1824 averaged 50.7 per cent of the initial dye concentration.

4. Following separate, simultaneous administrations of T-1824 and I.H.S.A. to ten subjects, removal and dilution of dye did not exceed the concomitant decline of plasma radioactivity by more than 12 per cent during one week of serial observations.

5. Disproportionate sampling has been suggested as a source of error in extrapolation techniques. Such disproportionate sampling can be anticipated if a labelled substance is mixed progressively throughout a series of compartments which differ in their intrinsic constants of equilibration and disposition.

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THE EFFECT OF A THYROTROPHIC HORMONE PREPARATION ON THE METABOLISM OF RADIOIODINE IN EUTHYROID, HYPERTHYROID AND ACROMEGALIC INDIVIDUALS [1]

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The role played by the thyroid stimulating hormone (TSH) of the pituitary in thyroid disease has excited speculation for some time. The similarity of the effects produced by this material to some of the physiological changes noted in Graves' disease seems more than coincidental. It was early shown that the injection of a crude anterior pituitary preparation in man would cause an elevation in the basal metabolic rate (1, 2). Later studies have shown that thyrotrophin administration to euthyroid normals will increase the radioiodine uptake of the thyroid and the level of the protein-bound iodine of the serum (3–5). Goldsmith, Stanbury, and Brownell have recently demonstrated that the injection of preparations of this material causes an increase in the rate of release of hormone from the thyroid gland (6). Studies on the rat and chick have demonstrated similar changes (7–10).

Evidence indicates that the circulating hormone of the thyroid is loosely bound to plasma proteins (11). Since this hormone is quantitatively precipitated with the proteins of the plasma (12) it is practicable, following the administration of a dose of radioiodine (I^{131}), to measure the output of thyroid hormone as protein-bound radioiodine. These techniques have been utilized in the present study to determine the effect of a single dose of thyroid-stimulating hormone on the discharge of labeled hormone by the thyroid. The consequences of the injection of this pituitary preparation are compared in euthyroid, hyperthyroid and acromegalic individuals.

METHODS

A test dose of I^{131} (as sodium iodide) of from 100 to 950 microcuries containing not more than 10 micrograms of carrier I^{127} was administered orally. The radioactivity in the serum, in the protein-precipitable fraction of the serum, in the urine, and over the neck was measured daily, and at more frequent intervals following thyrotrophin administration. Chemical measurements of the protein-bound iodine (PBI^{127}) were made using Barker's modification of Chaney's procedure (13). The protein-bound I^{131} was measured by precipitating aliquots of serum with zinc hydroxide in the same tubes in which the total serum activity was measured. Somogyi's method (14) was modified by doubling the strength of all solutions in order to reduce the volumes. Following three washings with distilled water, the radioactivity in the precipitate was determined in a "Texaco" gamma ray cup counter (15) (sensitivity—60,000 counts/minute/microcurie for I^{131}) to a minimum counting accuracy of 2 per cent standard error. Repeat determinations of radioactivity on 10 aliquots of the same serum had a standard deviation of 1.2 per cent of the mean. A standard deviation of 3.1 per cent of the mean was found, however, after these aliquots were precipitated and the radioactivity of the protein-bound I^{131} was determined. Recovery studies demonstrated that exchange between I^{131} and serum was negligible, as has been shown by McConahey, Keating, and Power (16).

The amount of radioactivity over the neck was determined with a well collimated "Texaco" Geiger tube 22 cm. above the suprasternal notch (15). The accuracy of this measurement will depend in part upon the geometry of the thyroid and was found to have a standard deviation of approximately 5 to 10 per cent of the mean in the same patient. The urine specimens were collected in daily 24-hour periods and the radioactivity of an aliquot was assayed by counting with a Geiger tube in a Marinelli beaker (15). All radioactivity measurements were corrected for the resolving time of the equipment involved and for physical decay of the isotope.

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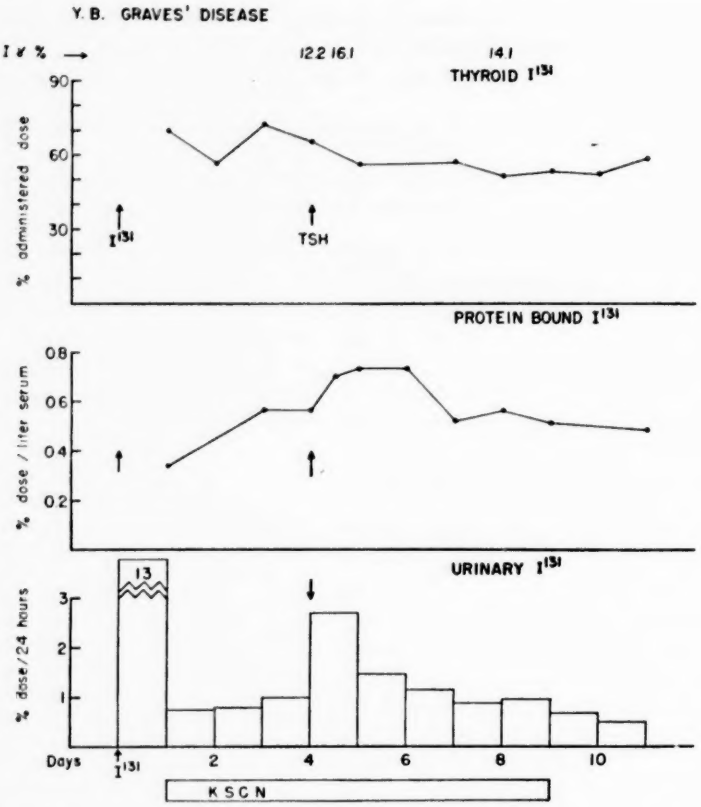

FIG. 1. THE EFFECT OF A SINGLE INJECTION OF TSH ON THE METABOLISM OF I^{131} IN A PATIENT WITH GRAVES' DISEASE

A pituitary preparation[4] (Armour) containing thyroid stimulating hormone was used in this study. The same amount (40 mg. equivalents) of the same preparation was used in 12 of the patients studied. The remaining two (E. G. and B. V.) received 10 mg. equivalents each of the same material. The lyophylized pituitary extract was dissolved before injection in a volume of 2 cubic centimeters of sterile saline and administered intramuscularly.

The experimental subjects were hospitalized (with the exception of S. B., M. L., and S. M.) and kept on a diet relatively low in iodine but otherwise unrestricted.[5] The euthyroid patients were patients with carcinoma of the breast (M. D. and R. M.), arteriosclerotic heart disease (R. B.) and malignant melanoma (F. F.). History, physical examination and the usual laboratory studies (PBI, radioiodine studies, BMR, cholesterol) confirmed their thyroid status. Laboratory examinations demonstrated that all patients had apparently normal renal function. All patients had been under observation in the clinics of either the Memorial or The New York Hospital for some time and their diagnoses were considered to be well established.

The procedure of the experiments was as follows: On the first day, I^{131} was given orally to the fasting pa-

[4] Armour Thyrotropic Factor, Control number J-13903-R. Each vial of this material was stated to contain 20 milligram equivalents of the Armour standard pituitary preparation. Actual weight of the contents of each vial was about 35 milligrams. This material was assayed in the laboratories of the Sloan-Kettering Institute by Dr. William Money to whom we are especially indebted. In this assay, day-old male chicks were injected daily for 3 days with various amounts of this preparation. The animals were killed on the fourth day and the weight of the thyroids determined. Comparison to a standard pituitary preparation assayed in the same manner demonstrated that 1 milligram (actual weight) of the preparation used contained 0.5 Junkman-Schoeller units.

[5] Only uniodized salt was used and foods known to contain large amounts of iodine (mainly sea food) were omitted.

tient. Twenty-four hours later 1 gram of potassium thiocyanate, in the form of enteric-coated tablets, was given orally to prevent the incorporation of further iodine into the thyroid (17). This drug was then continued in a dosage of 0.25 gram twice a day for the duration of the experiment. Serum thiocyanate levels were determined by the method of Gregersen and Stewart (18). When the protein precipitable radioactivity reached a plateau (4 to 8 days after the administration of the tracer) TSH was administered. Determinations were made of serum and neck radioactivity immediately before the injection of TSH and every 2 hours afterward for 8 hours. Eosinophile counts were done in several patients both before and 4 hours after the injection of the TSH.

RESULTS

Figure 1 shows the effects of a single injection of TSH in a patient with Graves' disease. Following the injection of thyrotrophin, no significant change occurred in the readings of radioactivity over the neck. By 24 hours after the injection, however, the PBI^{131} had risen 30 per cent and the PBI^{127} a similar amount.[6] There was a more marked effect on radioactivity measurements in the urine, with the 24-hour collection of urine following the TSH containing over 150 per cent more radioactivity than the 24-hour urine in the period preceding the injection. These same indices are compared in an euthyroid patient in Figure 2.

[6] The changes (in terms of percentage) in the various indices are calculated on the basis of a comparison with the "control" determination—that value immediately preceding the administration of the thyroid-stimulating hormone.

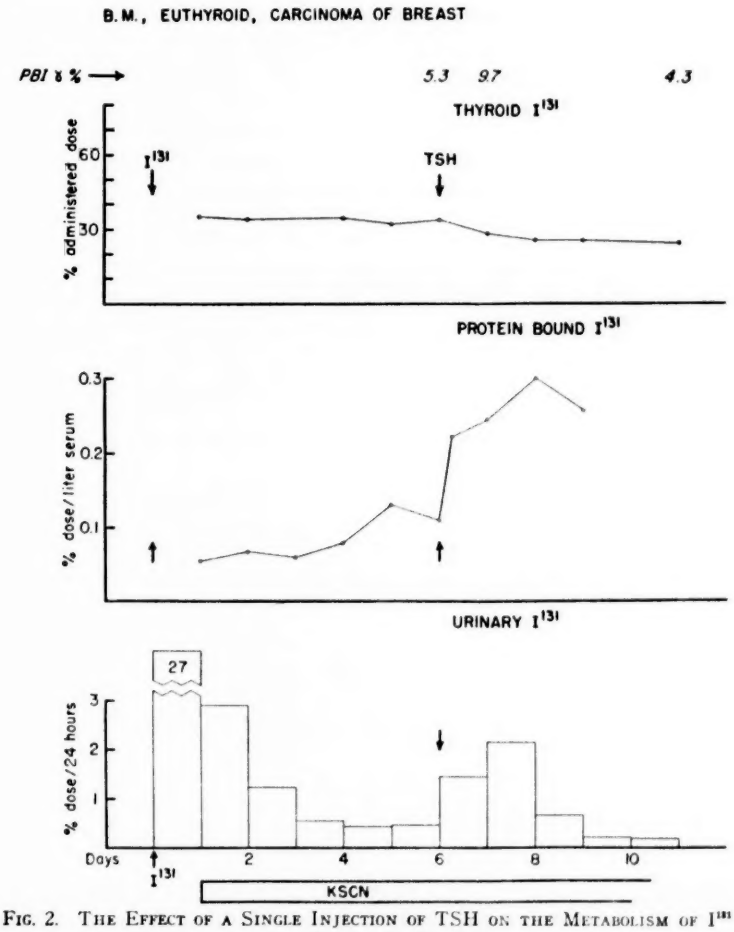

FIG. 2. THE EFFECT OF A SINGLE INJECTION OF TSH ON THE METABOLISM OF I^{131} IN AN EUTHYROID SUBJECT

TABLE I—*Total and precipitable I^{131} in the serum*

Patient		Day after I^{131} administration									Hour after thyroid stimulating hormone administration												
		1	2	3	4	5	6	7	8		2	4	6	8	12	24	48	72	96	120	144	168	
M. D.	[1]T.S. [2]PBI[131]	0.48 0.036	0.11 0.045	0.074 0.049	0.057 0.044	0.054 0.047	0.055 0.037	0.053 0.043	—* 0.069	Thyroid Stimulating Hormone 40 mg. equiv. I.M.	0.071	0.074 0.045	0.045	0.091 0.071		0.11 0.10	0.11	0.072			0.048		Euthyroid, carcinoma of breast
B. M.	T.S. PBI[131]	0.46 0.057	0.12 0.067	0.13 0.062	0.14 0.078	0.12 0.13	0.12* 0.11				0.15 0.16	0.20	0.25 0.22	0.30		0.38 0.25	0.30	0.34	0.26	0.20	0.25	0.20	Euthyroid, carcinoma of breast
R. B. (I)	T.S. PBI[131]	0.35 0.038	0.14 0.061	0.12 0.075	0.12 0.092	0.14*					0.18	0.24	0.30	0.28	0.28 0.23	0.33 0.28	0.31 0.27	0.26 0.24	0.25 0.23	0.22	0.19	0.17	Euthyroid, arteriosclerotic heart disease
J. M.	T.S. PBI[131]	0.17	0.26 0.25	0.38 0.36	0.40* 0.34						0.40 0.36	0.44 0.39		0.44	0.40	0.42	0.42 0.42	0.40	0.39 0.39				Graves' disease
J. G.	T.S. PBI[131]	0.21 0.19	0.32 0.29	0.44 0.42	0.51 0.49	0.54* 0.54					0.78 0.70	0.98 0.84	1.05 0.85	1.02 0.84	1.02 0.88	1.14 1.06	0.96 0.83	0.84	0.90 0.82	0.88 0.80	0.91 0.85		Graves' disease
[3]E. G.	T.S. PBI[131]		0.86 0.83	1.03 0.98	1.09* 1.02							1.37 1.20		1.51 1.35		1.47 1.41	1.25 1.16						Graves' disease
[4]B. V.	T.S. PBI[131]							0.55 0.40	0.50* 0.52			0.54 0.52		0.56 0.55		0.64							Graves' disease
Y. B. (I)	T.S. PBI[131]	0.36 0.34	0.51	0.64 0.57	0.60* 0.57						0.80 0.73		0.86 0.77	0.83 0.72	0.77 0.71	0.85 0.74	0.73 0.74	0.63 0.53	0.64 0.57	0.63 0.52	0.60	0.55 0.49	Graves' disease
[5]Y. B. (II)	T.S.	0.39	0.44	0.38	0.49*						0.49	0.57 0.58	0.57 0.57	0.58 0.57		0.62 0.60	0.58	0.58 0.59	0.56 0.52	0.54 0.49			Graves' disease
S. S. (I)	T.S. PBI[131]	0.16 0.14	0.35 0.28	0.39 0.37	0.37* 0.38						0.46 0.42	0.57 0.51	0.56 0.51	0.56 0.50		0.60 0.59	0.72 0.69	0.69 0.66	0.59 0.55	0.56 0.54		0.44	Graves' disease
[5]S. S. (II)	T.S. PBI[131]	0.14 0.15	0.22 0.23	0.29 0.30	0.39* 0.37						0.38 0.36	0.42 0.39	0.46 0.43	0.46 0.44		0.49	0.48	0.56 0.56	0.57 0.54	0.71 0.67	0.66 0.64	0.59 0.54	Graves' disease
M. L.	T.S. PBI[131]	2.57 2.43	3.03 2.83	2.99 2.90	3.03* 2.79							2.86 2.71		3.32 2.98		3.13 2.84	2.82 2.59	2.86 2.56	2.47 2.28	2.66 2.16			Graves' disease
H. D.	T.S. PBI[131]	1.05 1.06	1.76 1.72	1.83 1.77	2.05* 1.86						2.21 2.00	2.27 1.95	2.29 2.03	2.38 1.92	2.15 1.86	2.23 1.93	2.10 1.91	1.71 1.69	1.50 1.59				Nodular goiter, hyperthyroid (?)
P. O.	T.S. PBI[131]	2.91 2.84	3.23 3.05	3.06 2.83	2.80* 2.78						2.98 2.82	2.82 2.77		2.82 2.73	3.08 2.89	2.64 2.50	2.45 2.39	2.12	1.90		1.56		Acromegaly, Graves' disease
S. B.	T.S. PBI[131]	0.11 0.024	0.050	0.061 0.049	0.054 0.036	0.064 0.055	0.066* 0.067					0.11 0.077		0.19 0.089		0.14 0.12	0.13 0.14	0.11 0.10	0.11 0.096				Acromegaly, euthyroid
S. M.	T.S. PBI[131]		0.16 0.11	0.14 0.093	0.11 0.10	0.14 0.14	0.15*				0.12	0.17	0.19 0.18	0.15 0.15		0.20 0.17	0.21 0.17	0.19	0.17	0.14			Acromegaly, euthyroid
[6]R. B. (II)	T.S. PBI[131]	0.68 0.040	0.19 0.066	0.14 0.087	0.13 0.11	0.14* 0.11				Cortisone	0.15 0.13	0.13 0.12	0.13 0.12	0.13 0.11	0.15 0.13	0.17 0.13	0.18 0.15	0.16 0.15	0.16 0.15	0.16 0.15	0.16		Euthyroid, arteriosclerotic heart disease

Patient		Day after I^{131} administration												
		1	2	3	4	5	6	7	8	9	10	11	12	
[6]F. F.	T.S. PBI[131]	0.62 0.034	0.34 0.12	0.23 0.17	0.26 0.21	0.28 0.24	0.31 0.26	0.29 0.26	0.32 0.27	0.33 0.28	0.27	0.26	0.28	Euthyroid, malignant melanoma

All values are in terms of per cent of the administered dose of radioiodine per liter of serum. [1] T.S.—total serum radioactivity. [2] PBI^{131}—protein bound radioiodine.

In all experiments, except those labeled Y. B. (II) and S. S. (II), the patient was given 1.0 gm. of potassium thiocyanate 24 hours after the I^{131} was administered. This medication was continued at a dosage of 0.5 mg. per day for the duration of the experiment.

[3] 10 mg. equivalents of TSH was given in place of the usual 40. [4] Patients Y. B. (II) and S. S. (II) received no thiocyanate.

[5] 500 mg. of cortisone was given orally in place of the usual injection of thyroid stimulating hormone. [6] Neither TSH nor cortisone given.

* The asterisk indicates the value obtained immediately before the administration of the TSH. It thus indicates the day on which the medication was given.

Again, the pituitary preparation produced no effect on the amount of radioactivity measured over the neck. The serum PBI^{131} in this patient showed a more marked response to TSH. An elevation of 130 per cent was produced by the end of 24 hours, and by 48 hours this index had risen over 200 per cent of its "control" value. The PBI^{127} measured at 24 hours showed a rise of 80 per cent. The urinary excretion of I^{131} demonstrated a somewhat different pattern from that seen in the hyperthyroid patient in Figure 1. Here, more of the radioiodine was excreted in the second 24-hour period following TSH, with elevations of 310 and 325 per cent in the first and second 24-hour periods, respectively.

Table I lists all the determinations of the radioactivity in both the total serum and the protein-precipitable fraction of the serum. It can be seen that by 24 hours following the administration of I^{131} almost all the serum radioactivity of the hyperthyroid patients was protein bound. In the euthyroid patients however, the radioactivity did not completely precipitate with the proteins until 4 to 6 days had elapsed after the administration of the I^{131}. Because of this, the euthyroid patients were given TSH between the fifth and eighth day following the tracer dose whereas the hyperthyroid patients received the injection by the fourth day. It can also be noted in this table that the blood levels of radioactivity in the hyperthyroid patients tended to be somewhat higher than those of the euthyroid patients, as was previously noted by McConahey, Keating, and Power (16).

Within two hours after the injection of TSH many of the patients showed an elevation in the blood level of PBI^{131}. A maximum elevation was observed in the hyperthyroid patients within 24 hours after administration of TSH. Euthyroid patients, on the other hand, did not show a maximum response until 48 hours after the administration of TSH. In addition, elevations of the blood levels in the euthyroid patients were somewhat greater than those in the hyperthyroid subjects (Figure 3).

In Table II are listed the measurements of radioactivity in the neck (in terms of per cent of administered dose). With the possible exception of patient J. G., no patient demonstrated any decrease in this index that could be attributed to the administration of the single dose of thyrotrophic material. Although not appearing on this table, measurements were made 2, 4, 6 and 8 hours following the injection and these determinations likewise showed no significant changes.

The biological half-life of the radioiodine in the thyroid (observed half-life corrected for physical decay) ranged between 11 and 50 days with the exception of 3 patients (Table II). These patients (P. O., H. D., and M. L.), the only subjects showing insignificant response to the injected TSH, had an extremely rapid half-life of I^{131} in the thyroid (5.0, 2.5 and 6.3 days, respectively).

In Table III are shown values for the urinary excretion of radioiodine per 24-hour period in terms of percentage of the dose administered. These determinations fall into the usual patterns for euthyroid and hyperthyroid patients. Following administration of the TSH preparation, a considerable elevation in the urinary radioactivity was demonstrated in all except 3 of the patients studied. These patients (P. O., H. D., and M. L.), previously noted for their high turnover rates of

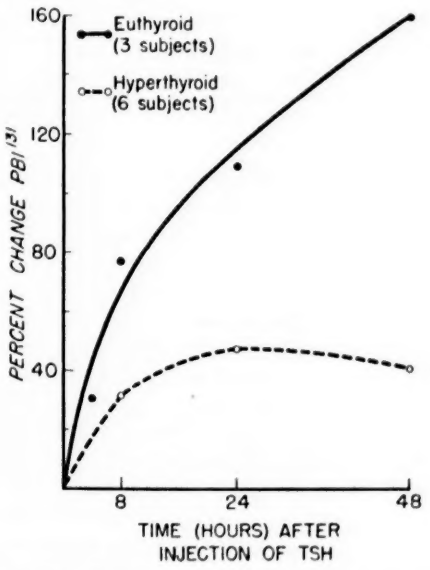

FIG. 3. THE EFFECT OF TSH ON THE LEVEL OF THE SERUM PBI^{131}

Values are calculated in terms of per cent increase in PBI^{131} as compared to a control value taken immediately before the injection of TSH.

TABLE II

Radioactivity measured over the neck

Patient	Dose I^{131}	Day after I^{131}									Day after TSH					Biological half-life of I^{131} in thyroid
		1	2	3	4	5	6	7	8		1	2	3	4	5	
	μc															*days*
Euthyroid																
M. D.	.594	16.4	21.1	20.4	19.3	19.8	17.8	20.0	15.9*		15.1	15.9	16.0	14.2		22
B. M.	.685	35.0	33.9		33.8	32.6	33.0*				28.2	25.1	24.9		23.7	18
R. B. (I)	.740	30.6	29.0	34.6	31.6	27.8*					24.6	25.8		25.0	25.2	31
Graves' Disease																
J. M.	.476	64.3		72.5	54.2*						53.3	61.7	58.6			20
J. G.	.917	68.6	74.7	72.4	68.1	73.4*					55.4	52.2	59.0		56.4	23
†E. G.	.500		72.5													
†B. V.	.600		65.2													
Y. B. (I)	.528	70.0	56.8	73.9	66.0*						57.1		58.0	52.1	53.9	38
†Y. B. (II)	.870	68.5	61.9		49.5*						48.0	43.7	51.2	56.0	53.3	16
S. S. (I)	.456	40.4	47.7		42.7*						35.7	33.2	28.9	30.0	28.5	11
S. S. (II)	.815	55.9	47.7		50.6*						39.6	43.3	42.5	42.0	39.7	16
M. L.	.100	49.2	43.0	37.3	34.9*						30.5	28.6		27.6	23.1	6.3
H. D.	.800	54.4	37.3	29.6	25.0*						15.5	11.3	10.0			2.5
Acromegaly																
P. O.	.820	44.7	30.8	24.1	19.9*						15.2	15.3	13.4	10.2		5.0
S. B.	.497	32.4	30.3	30.2	31.9		27.4				25.0	25.0	25.6	25.3		20
S. M.	.502	26.6	20.3	19.7	20.0	19.0*					17.1	14.9	16.5	16.7		27
†R. B. (II)	.950	20.7	22.0	22.6	22.8	22.6*				Cortisone	21.1	20.7	20.7	19.0	19.5	50

Control		Day 1	2	3	4	5	6	7	8	9	10
†F. F.	.644	32.4	32.5		26.6		26.0	26.4	26.3	24.0	24.0

All values are in terms of per cent of administered dose of radioiodine.
All other notations are the same as those on Table I.

radioiodine, will be considered as a separate group and are excluded from the general discussion of the effects of TSH. Their data are, consequently, not included in Figures 3 and 4. In terms of percentage increase over "control" values for the various indices, the effect of TSH was the greatest in the urine radioactivity measurements. Here, increments ranged from 100 to 350 per cent as compared to elevations in the serum PBI^{131} which ranged from 30 to 200 per cent. Again, it may be noted that the hyperthyroid patients reached their maximum increments within the first 24 hours following thyrotrophin injection while the euthyroid patients often excreted considerably more radioactivity in the second 24 hours. Figure 4 shows the changes in the average percentage increment in urinary I^{131} in the three 24-hour periods following injection of TSH.

Table IV shows the values for the serum protein-bound iodine determined chemically. These values show elevations of varying degrees following the administration of TSH. From these data and the PBI^{131}, it is possible to calculate the specific activity (the ratio of atoms of radioactive isotope to atoms of stable isotope) of the iodine of the blood before and after the administration of the thyrotrophin. These figures (Table V) remain relatively constant for each patient.

DISCUSSION

TSH has been previously shown to cause the secretion of thyroid hormone by the thyroid gland in animals and in patients both normal and hyperthyroid (3, 6, 8–10, 19). The present study differs from previously reported investigations in attempting to quantitate the variations in response to a standard dose of TSH given to normal, hyperthyroid and acromegalic man.

TABLE III

Urinary excretion of radioiodine

Patient	Day after I^{131}									Day after TSH						
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7
Euthyroid																
M. D.	62.0	9.89	1.65	.716	.515	.382	.291	.303*		1.25	1.28	.71	.421	.333	.252	.204
B. M.	26.9	2.90	1.22	.559	.442	.450*				1.47	2.13	.875	.210	.205	.178	.168
R. B. (I)	43.5	6.24	1.85	.900	.660*					2.08	2.88	1.02	.350	.340	.360	.320
Graves' Disease																
J. M.	5.18	.559	.895	.709*						1.69	.891	.825	.737			
J. G.	9.36	.574	.803	.952	1.04*					4.51	2.23	.924	.900	1.00	1.05	
[2]E. G.	10.5	.403	.386	.962*						2.58	1.85					
[2]B. V.	22.4	.790						.340*		.953	.360					
Y. B. (I)	12.8	.791	.802	1.04*					T S H	2.72	1.49	1.18	.905	.995	.700	.631
[4]Y. B. (II)	30.4	1.57	1.03	1.06*						2.95	1.95	1.11	1.11	1.67	1.20	
S. S. (I)	45.7	1.25	1.34	1.55*						2.66	(2.00)	2.18	1.26	1.06	.800	
[4]S. S. (II)	46.4	.918	.622	.645*						.995	.625	.591	.635	.385	.580	
M. L.	12.6	3.01	2.42	3.31*						4.35	2.32	2.26	1.74	1.23		
H. D.	24.8	5.95	5.90	4.70*						7.12	4.80	3.12	2.86	2.49	2.42	
Acromegaly																
P. O.	16.8	3.09	5.15	4.03*						3.95	4.04	3.57	3.16	3.29	2.44	2.43
S. B.	33.5	2.41	.630	.420	.360	.374*				1.33	.591	.185	.116			
S. M.	57.8	7.75	1.73	.360	.617	.526*				1.40	.990	.572	.273	.291		
[3]R. B. (II)	56.9	11.9	1.66	.851	.577*				Cortisone	.508	.390	.431	.378	.393	.412	

Control	Day 1	2	3	4	5	6	7	8	9	10
[3]F. F.	41.1	7.99	2.74	1.01	.910	.994	.973	.951	.949	.910

All values are in terms of per cent of administered dose per 24 hour period.
All other notations are the same as those on Table I.

TABLE IV

Protein-bound iodine (PBI^{127}) in serum
(All values in $\gamma\%$)

Patient	Control immediately before TSH	8 hours post-TSH	24 hours post-TSH	48 hours post-TSH	72 hours post-TSH
Euthyroid					
M. D.	5.4	6.9		9.6	
B. M.	5.3		9.7		
R. B. (I)	8.7	15.3	18.4		9.5
*R. B. (II)	8.3	5.2	3.3	5.8	
Graves' disease					
J. M.	17.8	18.9		19.8	
J. G.	11.4	15.0			
E. G.	15.3		18.7		
B. V.	20.5		24.6		
Y. B. (I)	12.2	16.1			
S. S. (I)	4.7	6.1		9.5	
H. D.	6.3	6.5		6.9	
M. L.	16.6	20.1	19.1	16.1	
Acromegaly					
P. O.	5.5	4.1			
S. B.	5.6		9.2		
S. M.	9.5	10.6	12.7	14.2	11.3

Control	Day 6	Day 9	Day 10
F. F.	3.5	3.0	2.8

* This patient received cortisone (500 mg.) instead of TSH.

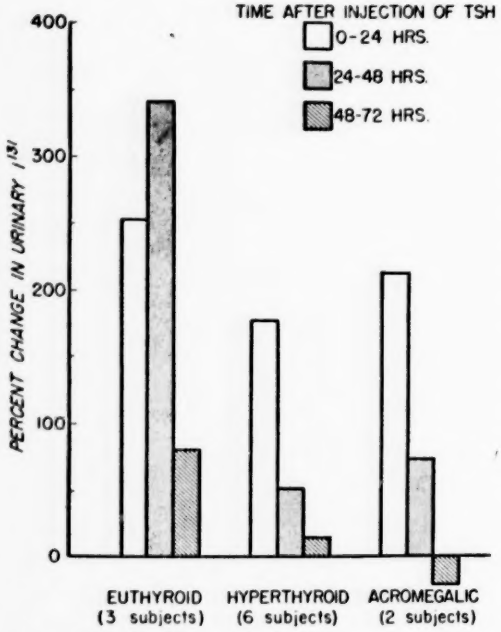

FIG. 4. THE EFFECT OF TSH ON URINARY EXCRETION OF RADIOIODINE

Values are calculated in terms of per cent change in 24-hour urinary excretion as compared to the "control" 24-hour period immediately prior to the administration of the TSH.

The negative value seen on the third day in the acromegalic patients represents an average value below that obtained on the control day.

Certain difficulties are inherent in the use of TSH at the present time and in the quantitation of this study. The preparation of TSH used was obviously impure and may have been contaminated with small amounts of other pituitary hormones. Since there was no change in the volume of urine secreted before and after the administration of TSH, it appears unlikely that antidiuretic hormone was present in sufficient amount to interfere with the study. Eosinophile counts were performed before and 4 hours after the TSH to evaluate the possibility that ACTH was a major contaminant. In three of five patients studied there was no significant change in eosinophile count after TSH but in two patients there was a significant fall. A further control was done to see if adrenocortical hormones whose secretion might have been caused by ACTH would affect the secretion of thyroid hormone. In one patient (R. B.–II) 0.5 gram of cortisone was administered orally at the time TSH was usually given. No change was seen subsequent to this in the serum, urinary, or thyroidal radioiodine. Another possibility is that TSH (or contaminants) might affect the rate of breakdown, excretion, or distribution of thyroid hormone. In one patient TSH was administered 10 days after intravenous injection of thyroxine labeled with I^{131}. There was no change in the level of radioactivity in the blood or urine that could be attributed to TSH.

Thiocyanate might have a specific effect on the response of the thyroid to TSH. In two hyperthyroid patients (Y. B.–II and S. S.–II) the usual experimental protocol was repeated except that the administration of potassium thiocyanate was omitted. Only random deviations were noted in these experiments indicating that the thiocyanate had no specific effect on the response of the gland to thyroid-stimulating hormone. Since iodide released upon de-iodination of the thyroid hormone is in part accumulated by the thyroid, one would expect a greater urinary excretion of iodine if thyroidal reaccumulation is prevented (as by thiocyanate). This occurred with S. S. but not with Y. B. (Table III).

TABLE V

Iodine in thyroid and iodine discharged by TSH

Patient	Iodine discharged subsequent to TSH	Estimated I^{127} in thyroid	Thyroidal iodine discharged by TSH
	mg.	*mg.*	*%*
Euthyroid			
M. D.	2.27	12.4	18.3
B. M.	2.95	15.9	18.6
R. B.	4.31	22.0	19.6
Graves' disease			
J. M.	1.17	28.4	4.12
J. G.	1.60	15.7	10.2
E. G.	0.544	—	—
Y. B. (I)	0.881	14.1	6.18
S. S. (I)	0.804	5.3	15.1
*H. D.	—	0.85	—
*M. L.	—	2.1	—
Acromegaly			
*P. O.	—	0.39	—
S. B.	1.81	22.9	7.91
S. M.	1.09	12.9	8.45
Control			
F. F.	—	2.5	—

* Patients H. D., M. L., and P. O. did not respond to administered TSH.

Another possible source of error is failure of thiocyanate to inhibit iodine uptake by the thyroid. The interpretation of the data is contingent upon the assumption that there is no recirculation of iodine from the catabolized thyroid hormone back into the thyroid. Thiocyanate levels were usually maintained at 4 to 5 milligrams per cent. However, somewhat lower levels of thiocyanate appeared to be partially effective. In one patient (B. M.) there was 35 per cent uptake of a tracer dose of I^{131} which was depressed to 7 per cent uptake where the level of thiocyanate was 1.8 milligrams per cent. Another tracer showed 4.8 per cent uptake when the level of thiocyanate in the blood was 3.1 milligrams per cent. It should be noted that even in an athyreotic patient the radioiodine in the tissues of the neck gives an apparent uptake of about 2 per cent. If the uptake of iodine is not inhibited, the level of radioiodine in the precipitable fraction of the serum rises, and by 4 to 10 days falls again. If, on the other hand, recirculation of iodine is prevented, the level of precipitable I^{131} in the serum will increase asymptotically and not fall until the entire store of iodine in the thyroid is exhausted. In control studies (F. F., R. B.–II) it is apparent that the level of PBI^{131} reaches a plateau and does not fall. This is fairly conclusive evidence that the recirculation of iodine was prevented by the thiocyanate.

If the recirculation of radioiodine has been prevented by thiocyanate then the specific activity of the precursor, which in this case is thyroglobulin stored in the thyroid, is maintained constant. Zilversmit has described mathematically the sequence of events in the two situations when the specific activity (S. A.) of the precursor varies and when it is constant (*20*). In the first case the specific activities of the precursor and product are identical at only one point, the maximum S.A. of the product. In the second case, however, the S.A. of the product approaches asymptotically the S. A. of the precursor. As can be seen in the two control studies (F. F. and R. B.–II) the rate of increase in the S.A. of the product is quite rapid so that by 4 to 7 days after administration of the tracer a plateau is reached for all practical purposes. At any time thereafter it is possible to calculate iodine in the thyroid since the S.A. of the product and the activity of the precursor are known. These data are shown in Table V. If any iodine in the thyroid is in a metabolically inactive pool and therefore is not labeled by the I^{131}, the values calculated for thyroidal iodine will be too small by a factor related to the size of the inert iodine pool.

In general it appears that euthyroid patients have somewhat more iodine stored in their thyroid than do hyperthyroid patients. Patient J. M. had been treated with Lugol's solution intermittently for 10 years prior to the present study and it is not surprising to find, therefore, that there appeared to be a larger amount of iodine in his thyroid. The average values for both euthyroid and hyperthyroid patients are somewhat greater than those found on actual analysis of total iodine content of thyroids as determined by Gutman, Benedict, Baxter and Palmer, who found an average of 8.8 milligrams in normal thyroids and 5.1 milligrams in thyroids of hyperthyroid patients who had not been treated with iodine (*21*). No explanation is immediately apparent to explain this discrepancy.

From the time of plateau of the serum PBI^{131} until exhaustion of iodine stores in the thyroid a steady state prevails with respect to both the I^{127} and I^{131} in the serum. One would expect, therefore, that the specific activity of the serum would remain relatively constant. That this did occur is seen in Table VI and a measure of the constancy in S.A. for each patient is the fact that the average deviation of these values from their own mean is 9 per cent. Since the steady state lasts for at least a week it is possible to calculate the magnitude of an increment in serum PBI^{127} and PBI^{131}, if this is of relatively short duration. For calculation of the total increment, however, the volume of distribution of the PBI^{131} at the time of calculation must be known. Albert and Keating have shown that by 48 hours after intravenous administration of thyroxine labeled with I^{131} the early rapid disappearance into the volumes in which it is distributed is essentially complete. At this time the volume of distribution is approximately 20 per cent of the body weight (*22*). This figure can be used in calculating the volume of distribution of the PBI^{131} 48 hours after TSH. A certain fraction of the hormone discharged by TSH will, in 48 hours, have been excreted in the urine. Since the serum is the only source of urinary iodine the S.A. of serum and urine are as-

TABLE VI

Specific activity of protein-bound iodine in serum
$\mu c/\gamma$

Patient	Control	Hours after TSH injection 8	24	48	72	96	144
Euthyroid							
M. D.	0.0076	0.0061		0.0086			
B. M.	0.014	0.030	0.018				
R. B. (I)	0.0094	0.012	0.011				
*R. B. (II)		0.020	0.023	0.024			
Graves' disease							
J. M.	0.0091			0.010			
J. G.	0.043	0.0513					
E. G.	0.033		0.038				
B. V.	0.015		0.018				
Y. B.	0.025	0.024				0.021	
S. S.	0.037	0.037		0.033			
H. D.	0.25	0.23	0.28				0.29
M. L.	0.016	0.015	0.016	0.018			
Acromegaly							
P. O.	0.41	0.55					
S. B.	0.0060		0.0065				
S. M.	0.0079	0.0071	0.0079	0.0074	0.0084		

Control	Days after tracer 9	10	14
F. F.	0.060	0.062	0.064

* This patient received cortisone instead of TSH.

sumed to be identical. Then the following equation gives an approximate value for the iodine discharged by the single injection of TSH (over and above that which was normally secreted).

$$I^{127} = \left(\frac{\Delta PBI^{131} \times cPBI^{127}}{cPBI^{131}}\right) \times .2\,BW + \left(\frac{\Delta UI^{131} \times cPBI^{127}}{cPBI^{131}}\right)$$

where I^{127} = iodine discharged during the 2 days subsequent to TSH. This does not include the iodine secreted at the previously established rate for that patient at that time,

ΔPBI^{131} = increment in PBI^{131} of serum 48 hours after TSH in per cent of dose per liter of serum,

$cPBI^{127}$ = PBI^{127} in serum prior to TSH in micrograms per liter of serum,

$cPBI^{131}$ = PBI^{131} in serum immediately before the injection of TSH in per cent per liter of serum,

BW = body weight in kg.,

ΔUI^{131} = total increase in I^{131} in the urine during the 48 hours after the TSH in per cent of dose.

The amount of iodine discharged from the thyroid in response to a single injection of TSH as calculated above is shown in Table V. It is apparent that the euthyroid patients secreted more iodine in response to the same amount of TSH than did the hyperthyroid patients. The patients with acromegaly were intermediate between these two groups. It is of some interest, furthermore, that the hyperthyroid patients secreted a smaller proportion of iodine stored in their thyroid than did the euthyroid patients. If the patients with hyperthyroidism had increased levels of circulating TSH it would appear reasonable for a standard dose of TSH to produce less effect in these patients than in those who had a normal level of circulating TSH. Certainly such an explanation is little more than speculation without more direct data.

An additional feature of interest in which hyperthyroid and euthyroid patients differed was in the rapidity of response to TSH. As can be seen in Figures 3 and 4 the effect of TSH was not maximal in euthyroid patients until 48 hours after its administration, whereas in hyperthyroid cases the maximum effect was reached in 24 hours.

There were three (P. O., H. D. and M. L.) who showed no response to TSH. These patients, however, had such a small amount of iodine stored in the thyroid that were 10 per cent of it secreted in response to TSH (the average percentage of thyroidal iodine released from this

dose of TSH was 9 per cent for hyperthyroid patients) the increment in urinary or serum iodine would have been very difficult to detect. One cannot therefore consider these patients to have been unequivocally non-responsive to TSH. However, the pattern of iodine metabolism in these cases was quantitatively markedly different from that in the other patients. All three had very low thyroidal iodine, an unusually fast disappearance rate of iodine from the thyroid (6.3, 2.5 and 5.0 days) and abnormally elevated levels of PBI^{131} in the serum (2 to 3 per cent of dose per liter). These cases might represent a basically different physiology from the other cases of Graves' disease studied.

The patients with hyperthyroidism had varying degrees of exophthalmos from J. B. and H. D. who had none, to S. S. whose exophthalmos was very severe and who had recurrent corneal ulcerations. There appeared to be no correlation between the degree of exophthalmos and any of the parameters of iodine metabolism studied.

There is no clear explanation for the relatively greater increase in urinary I^{131} after TSH than in serum PBI^{131} or total I^{131}. At least two possibilites arise: *1*) Thyroid hormone and its iodine-containing metabolites are cleared by the kidney at rates dependent on their concentration in serum, or *2*) after TSH there is a relative increase in concentration in the serum of an iodine-containing compound which is rapidly cleared by the kidney. There are no data available bearing on this matter.

CONCLUSIONS

1. The effect of a single intramuscular injection of thyroid stimulating hormone on the metabolism of a tracer dose of I^{131} was studied in three euthyroid, eight hyperthyroid and three acromegalic patients.

2. When administered four to eight days after the tracer of I^{131}, TSH increased the level of PBI^{131} and PBI^{127} in the blood and I^{131} in the urine.

3. Methods are presented for the estimation of the total amount of thyroid hormone discharged from the thyroid after the injection of TSH and for calculation of the total iodine content of the thyroid.

4. Following a standard dose of TSH, euthyroid patients appeared to secrete more thyroid hormone than did hyperthyroid patients. The maximum effect of TSH appeared in the first 24 hours in the hyperthyroid patients, and not until 48 hours in euthyroid patients.

5. Three hyperthyroid patients are presented who demonstrated an unusual method of handling radioiodine. This is characterized by a very rapid turnover rate of iodine by the thyroid with consequent abnormally elevated serum protein-bound radioiodine and urinary radioiodine levels.

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A METHOD FOR DETERMINING CARDIAC OUTPUT BY THE DIRECT FICK PRINCIPLE WITHOUT GAS ANALYSIS[1, 2]

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With the reintroduction of cardiac catheterization (1) which facilitated the withdrawal of mixed venous blood for gas analysis, the Fick principle has been widely applied for the determination of cardiac output in man (2, 3). While this method provides the most accurate measurement of the cardiac output available at present and has contributed considerably to our knowledge of cardiovascular dynamics in health and disease, it is open, under certain circumstances, to several potential sources of error.

Chief among these is the requirement of gaseous equilibrium between the alveoli, blood and tissues. Any change in the alveolar gas content during the collection of exhaled air may produce not only significant error in the apparent oxygen consumption, but possibly changes in the arterial-mixed venous blood (A-MV) oxygen differences as well. Consequently, variations in respiratory function occasioned by anoxia, hyperventilation, exercise or inhalation of oxygen may lead to significant errors in the measured cardiac output. Since reestablishment of hematopulmonic equilibrium, or the steady state, following a change in respiratory function may require forty to sixty minutes (4), the immediate or early effects of altered respiratory dynamics on cardiac output cannot be evaluated.

Furthermore, the measurement of cardiac output by gas methods involves special equipment and time-consuming analyses of gases in both blood and respired air. In most laboratories, therefore, only a limited number of measurements are possible in a given period, and the separate determinations must be intermittent rather than continuous. Moreover, in some subjects, the periodic application of a face mask or nose clamp may produce anxiety, and thereby influence the measured cardiac output.

In order to overcome these difficulties, the possibility of using some non-gaseous test substance for measuring the cardiac output by the Fick principle was considered. The ideal substance for this purpose should possess the following characteristics: *1*) rapid, fairly constant and easily measurable bodily uptake and/or excretion of sufficient magnitude to produce a significant A-MV difference; *2*) ease and accuracy of determination in small quantities of body fluids by ordinary analytical methods; *3*) known or readily verifiable volume of distribution; and *4*) absence of toxicity and effects on circulatory dynamics.

When the cardiac output and the rate of removal of the test substance are constant, the magnitude of the A-MV difference is a function of two factors—the proportion of the cardiac output perfusing the extracting organ(s) and the completeness of extraction. Since the kidneys normally receive a large fraction of the cardiac output (5), any substance completely extracted by the kidneys would exhibit an adequately large A-MV difference. Moreover, renal uptake of blood constituents can be accurately measured by the urinary excretion. Sodium para-aminohippurate (PAH), which fairly well fulfills the above criteria, has a renal extraction of about 90 per cent (6). Since the normal renal fraction of the total cardiac output is about 20 per cent, the resulting anticipated A-MV difference of PAH would be 18 per cent (0.20×0.90) of the arterial concentration.

In these studies, therefore, PAH was first used; subsequently, to obviate difficulties arising from

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[2] A preliminary report was presented at the meeting of the Federation of American Societies for Experimental Biology, New York, April 17, 1952.

acetylation of a small fraction of the PAH (7), its acetylated derivative, para-acetylaminohippuric acid (PACA), which has the same renal clearance, was substituted. This was prepared as recommended by Newman and coworkers (8), the solutions being sterilized by passage through a Seitz filter.[3]

The use of a non-gaseous test substance presented a special problem. Unfortunately, the usual type of infusion into a peripheral vein cannot produce an A-MV difference, since under such circumstances, the concentration of test substance in the right side of the heart is as high or even slightly higher than in arterial blood. Only when the test substance is added to the blood of the pulmonary circulation, as is oxygen, can there result an A-MV difference. It is necessary, therefore, to introduce the test substance into the lesser circulation distal to that point from which mixed venous blood is withdrawn. Thus, if the test substance were added to the pulmonary artery and mixed venous blood removed from the right ventricle (in the absence of pulmonary regurgitation, interventricular septal defect, or removal in the lesser circulation), a true A-MV difference will be obtained regardless of the site of removal of the test substance. This was accomplished by placing the tip (distal lumen) of a double lumen cardiac catheter (9) into the pulmonary artery. The proximal lumen, used for the withdrawal of mixed venous blood, was located 10 cm. back, in the outflow tract of the right ventricle just below the pulmonic valve. In Figure 1, the placement of a double lumen catheter for the determination of an A-MV difference is diagrammatically represented.

[3] The authors are grateful to Miss J. Olch for her cooperation in filtering the preparations and testing the filtrates for sterility.

DERIVATION OF FORMULAE

The mathematical validity of the present application of the Fick principle (10) to the determination of cardiac output is established by the following derivation of the formulae used in the calculations.

The quantity of a substance circulating in a given portion of the blood flow is represented by the product of its concentration and volume of flow. In the case of a substance removed from the blood only by the kidneys, the concentration after equilibrium between blood and tissues has

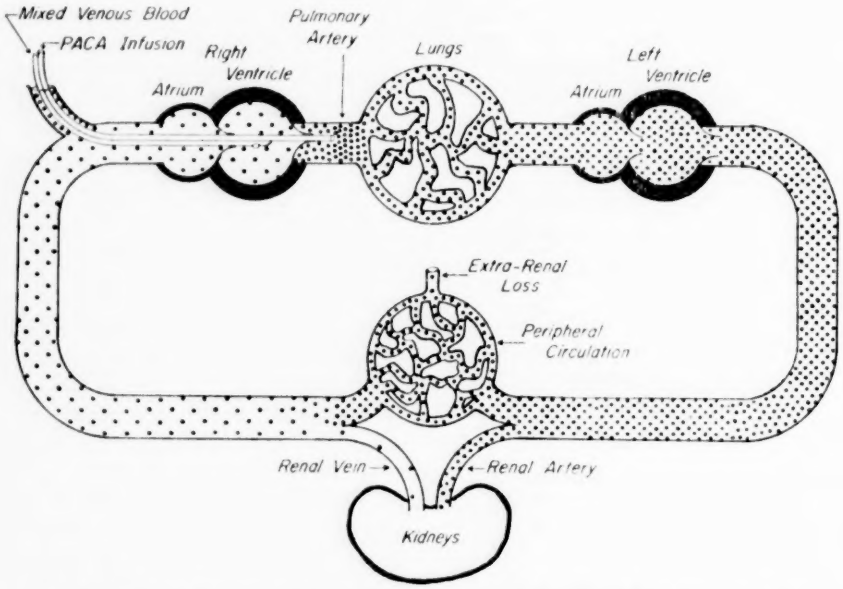

FIG. 1. DIAGRAM ILLUSTRATING THE PRODUCTION OF AN ARTERIAL-MIXED VENOUS BLOOD DIFFERENCE BY MEANS OF A DOUBLE LUMEN CARDIAC CATHETER

been achieved will be the same in all vessels of the greater circulation except those veins draining the kidneys (the renal veins and inferior vena cava). Under these circumstances, the amount of substance returning to the heart per unit time, the product of mixed venous blood concentration and total body flow (cardiac output), will be the sum of that returning from the kidneys (the renal venous blood concentration times renal blood flow) plus that returning from the remainder of the body. The latter quantity may be expressed as the product of the arterial concentration and difference between the cardiac output and renal blood flow. The above may be summarized for PACA as in the following equation:

1. $$[PACA]_{MV}(CO) = [PACA]_{RV}(RPF) + [PACA]_{A}(CO - RPF),$$

where

$[PACA]_{MV,RV,A}$ represent the concentration of PACA in mixed venous, renal venous and arterial plasma, respectively,

CO = cardiac output, as plasma,

RPF = renal plasma flow.

Transposing, and solving for CO,

2. $$CO = \frac{(RPF)([PACA]_{A} - [PACA]_{RV})}{[PACA]_{A} - [PACA]_{MV}}.$$

Since

3. $$[PACA]_{RV} = (1 - E)[PACA]_{A},$$

where E = renal extraction of PACA,

$$\left(= \frac{[PACA]_{A} - [PACA]_{RV}}{[PACA]_{A}}\right),$$

Substituting in equation 2 yields

4. $$CO = \frac{(RPF)([PACA]_{A} - (1 - E)[PACA]_{A})}{[PACA]_{A} - [PACA]_{MV}}$$

5. $$= \frac{(RPF)(E)[PACA]_{A}}{[PACA]_{A} - [PACA]_{MV}}.$$

But since

6. $(RPF)(E) = C_{PACA}$, the renal clearance of PACA, which $= \frac{UV_{PACA}}{[PACA]_{A}}$, where UV_{PACA} is the urinary excretion per unit time; therefore, substituting in equation 5,

7. $$CO = \frac{\frac{UV_{PACA}}{[PACA]_{A}} \times [PACA]_{A}}{[PACA]_{A} - [PACA]_{MV}} = \frac{UV_{PACA}}{[PACA]_{A} - [PACA]_{MV}}$$

which is the expression of the Fick principle for the measurement of cardiac output by the present method.

The above derivation is based upon the assumption that the removal of PACA from the plasma occurs only by renal excretion. In fact, PAH, and presumably PACA, is excreted by way of sweat (11) and other extra-renal channels, including the liver (12). Therefore, the following modification was introduced in order to express total plasma clearance. During the continuous infusion of a substance, at constant arterial concentration, the total amount cleared from the plasma by all routes must equal the amount administered. Under these circumstances, equation 7 may be expressed as follows:

8. $$CO = \frac{IV}{[PACA]_{A} - [PACA]_{MV}}$$

in which

I = concentration of PACA in the infusion (mg./ml.),

V = rate of administration of the infusion (ml./min.), and the product is the amount of substance infused per unit time (mg./min.).

When, because of altered cardiac output or renal hemodynamics, the steady state is disturbed and arterial concentration is not constant, a correction factor for the changing plasma level must be introduced into equation 8. The rate of change, or slope of the line representing arterial concentration, $d[PACA]_{A}/dt$ (mg./ml./min.), is a function of the difference between the rates of plasma infusion and plasma clearance. When this is multiplied by the volume of distribution (V_D) of the test substance, the product represents the difference between the amounts of test substance infused into and removed from the blood. Thus,

9. $$CO = \frac{IV \pm d[PACA]_{A}/dt(V_D)}{[PACA]_{A} - [PACA]_{MV}}$$

during falling (+) and rising (−) levels, respectively.

In practice, the A-MV difference employed is one integrated throughout the desired period rather than that based upon a single pair of determinations.

The above equations have all been expressed in terms of plasma. Conversion to whole blood figures is accomplished by dividing by (1-hematocrit).

MATERIAL AND METHODS

The eighteen subjects in this series included seven without cardiac or renal disease and eleven cardiac patients, ten of whom had rheumatic heart disease with mitral stenosis.

The subjects were in the post-absorptive state, at times mildly sedated with a barbiturate. A soft rubber, multi-holed urethral catheter was inserted, and an indwelling arterial needle placed into a femoral or brachial

artery. The double-lumen cardiac catheter was then inserted, and passed well into a branch of the pulmonary artery. A Sanborn electromanometer was connected to the proximal lumen, and the catheter slowly withdrawn during continuous recording to slightly beyond that point at which the transition from the pulmonary arterial to the right ventricular pressure tracings was observed. The proximal lumen now was located about 2 cm. below the pulmonic valve in the outflow tract of the right ventricle. Through the distal lumen (tip), which lay about 8 cm. into the pulmonary artery, the solution of the test substance was infused by means of a Bowman constant infusion pump at a rate of 1.0 to 3.5 ml. per minute.

Following a period of about one hour to permit equilibration of the test substance, specimens of arterial and mixed venous blood (right ventricle) and urine were obtained periodically for the continuous measurement of cardiac output by the chemical [4] method. At the same time, expired air and similar blood specimens were collected for the determination of cardiac output by the usual gas [4] method; the latter, however, were less frequent and intermittent. In those patients able to tolerate such activity, additional measurements were made following exercise.

Blood for oxygen determinations was collected in syringes with dead spaces obliterated by bubble-free heparin solution, and kept over mercury at 0° C. until analyzed by the Van Slyke and Neill manometric technique (13). Duplicate determinations were required to check within 0.1 volume per cent. Expired air was collected in a Tissot spirometer, transferred to tonometers, and analyzed by the Scholander respiratory gas method (14).

PAH was determined by the method of Smith and his associates (7). PACA was measured at first by the method of Newman (8); subsequently, the acidified filtrate was hydrolyzed in 25 ml. volumetric flasks and diluted to constant volume after completing the color reaction. Hematocrits were determined on arterial blood in Wintrobe tubes centrifuged at 3,000 RPM for thirty minutes. No correction was made for trapped plasma.

RESULTS

Certain conditions and assumptions underlie the successful application of the chemical method. Firstly, competency of the pulmonic valve, particularly with the catheter *in situ*, should be established. Observations on the present series of patients have confirmed the reports of others that no alterations in the recorded pressure curves occur when double lumen catheters are used. In addition, to test roughly whether regurgitation occurs, Evans blue dye (T-1824) was injected into the pulmonary artery via the distal lumen of a catheter, and blood was simultaneously withdrawn from the right ventricle. No dye was detectable in the right ventricular blood during an interval shorter than the circulation time, including that of the coronary circulation.

Secondly, success of the method requires complete mixture in the blood during passage of the test substance from the pulmonary artery to the aorta.[5] Although streaming may tend to prevent complete admixture, the relatively small volume of test solution added to the large volume of blood, the complete passage through a capillary bed, and further mixing in the left chambers of the heart would favor homogeneity of the test substance throughout the arterial tree.

At first, the cardiac output was calculated from the urinary excretion of PAH, employing equation 7 which is based upon the assumption that the urinary excretion of PAH reflects the total plasma clearance. However, PAH has been found in other body secretions (11, 12). Moreover, in our earlier studies, the cardiac output, calculated from renal excretion of either PAH or PACA, tended to be about 15 per cent less than that obtained from gas analyses. That this discrepancy reflects extrarenal plasma clearance is supported by the fact that, during constant arterial concentrations, the infusion rate exceeded the urinary excretion by about this fraction. For this reason, the cardiac outputs here reported have been calculated by the use of equation 9, or the total plasma clearance, on the basis of the rate of infusion of the test substance.

The results are summarized in Table I and Figure 2. In general, the renal plasma flows and cardiac outputs conform with those previously reported (15). Among the rheumatic patients with mitral stenosis, both the circulatory and the renal hemodynamics, as well as the respiratory data, are in good agreement with those of Werkö and his colleagues (16, 17). Thus, the clearances of PAH and PACA in the cardiac patients are about one-third of those of the non-cardiac subjects. Moreover, the decrease in renal blood flow and in

[4] The terms "chemical" and "gas," as employed in this paper, are intended simply to differentiate the two methods, since the chemical nature of each is obvious.

[5] The same assumption is inherent in the dye or isotope dilution methods, especially since rapid injection is made. Actually, the lower molecular weight of PACA and slow rate of injection may be expected to result in more rapid mixing.

renal fraction of the cardiac output bears a rough relationship to the clinical estimate of the severity of failure. The oxygen consumption at rest is equivalent in both groups, but the oxygen uptake per liter of air ventilated is lower in the cardiac patients, a reflection of the respiratory dysfunction.

The cardiac outputs for corresponding collection periods indicate good agreement between the two methods. (Correlation coefficient, r = 0.90.) After use of the constant infusion pump resulted in more constant arterial levels, even better agreement was attained.

In one patient, G. M., a 26 year old male with sarcoidosis and pulmonary fibrosis, the effect of changing the gas content of the respired air on the determination of cardiac output by the gas and chemical methods was studied. Following control measurements while breathing from a tank of compressed air, a moderate degree of hypoxia was produced by permitting the subject to inhale a mixture of 10 per cent oxygen and 90 per cent nitrogen for 51 minutes. During this interval, the cardiac output was measured twice by the gas method and continuously by the chemical method (three urinary collection periods). Following return to the breathing of the air from the tank, additional measurements were made. The results are indicated in Figure 3.

Although there was generally good agreement between the two methods, certain differences were observed. During the hypoxic period, the output, as determined by the chemical method, rose initially to a maximal value of 12.1 l./min. then fell gradually to its previous level. At the same time, the cardiac output by the gas method rose more slowly so that the higher value, 11.05 l./min., was obtained toward the end of the hypoxic period. Following thirty minutes of inhalation of ambient air, the cardiac outputs, as measured by both methods, were at control levels.

The substitution of PACA for PAH was based upon previous reports (7, 8) that a small, but variable, fraction of the PAH is acetylated in the body. Such acetylation, contributing to extra-renal loss, would produce an error in the cardiac output as calculated from urinary excretion. In several studies, PAH was infused and the plasma and urinary concentrations of both PAH and PACA were determined by analyzing the plasma filtrates and diluted urine specimens both prior to and following hydrolysis. Although no significant amounts of PACA were present in arterial blood, the urine of these patients contained substantial amounts of the acetylated compound, sufficient, in fact, to make it appear that renal acetylation of

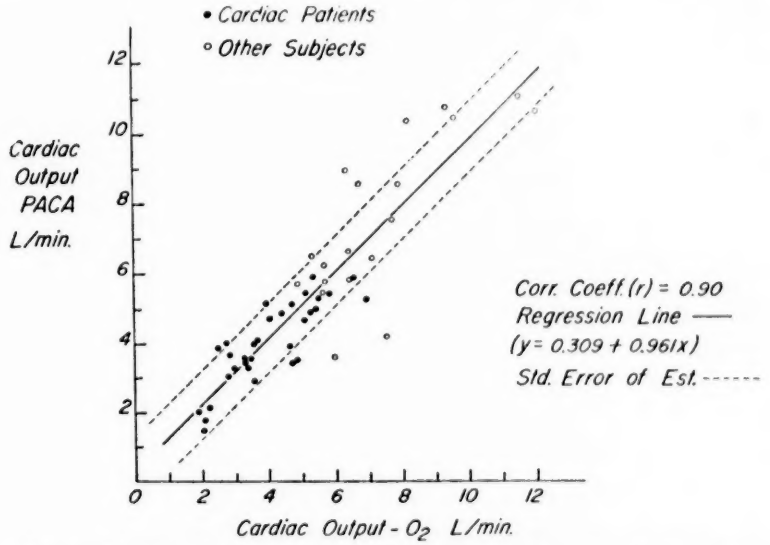

FIG. 2. CORRELATION BETWEEN THE CARDIAC OUTPUTS AS DETERMINED BY THE GAS (OXYGEN) AND CHEMICAL (PACA) METHODS

TABLE I

Summary of data

Patient Diagnosis, B.S.A.*	Sex, age	Period	Duration O_2	Duration PACA	C_{PACA}	O_2 cons.	O_2 cons. l. ventil.	A–V diff. O_2	A–V diff. PACA	Cardiac output O_2	Cardiac output PACA†
			min.	*min.*	*ml./min.*	*ml./min.*	*ml./l.*	*ml./l.*	*mg./l.*	*l./min.*	*l./min.*
Non-cardiac patients											
M. I.	F.	1	3	21.0	492	245	25.5	38.6	2.5	6.35	6.62
Rheumatoid arthritis	44	2	3	31.0	423	234	21.1	41.5	2.7	5.64	6.22
1.44		3		13.0	652				2.2		7.05
		4	3	32.0	536	204	20.5	38.6	2.3	5.29	6.54
S. W.	M	1	4	16.0	520	254	41.9	31.3	1.0	8.10	10.4
Pulmonary tuberculosis and fibrosis	42	2		10.5	523				1.9		10.2
1.71		3	7	20.0	499	268	42.3	40.4	2.05	6.62	8.61
		4		10.5	503				1.71		7.99
		5		13.0	499				1.67		9.70
		6	6	12.0	519	244	37.7	39.2	1.90	6.23	8.97
		7		17.0	510						7.88
M. S.	F	1	4	28.5	336	219	36.0	39.0	0.8	5.62	5.50
Cervical myelopathy	41	2	4	29.5	393	193	37.6	39.6	0.9	4.88	5.77
1.51		3	6	35.0	458	170	34.7	29.7	1.0	5.71	5.81
T. S.	M	1		19.0	649				3.0		7.79
Pulmonary fibrosis	50	2	6	26.0	583	296	31.8	38.4	3.21	7.70	7.58
1.85		3	4	23.0	593	304	30.6	38.9	2.76	7.82	8.61
M. V.	F	1		36.0	542				1.10		6.64
Possible hyperthyroidism	33	2	6	36.0	523	175	37.3	27.6	1.06	6.35	5.84
1.52		3		29.0	641				1.05		7.02
		4		14.0	580				1.02		6.82
		Exercise									
		5	4	16.0	510	212	45.9	30.0	0.93	7.07	6.44
		6		22.0	597				1.16		6.02
A. C.	M	1		44.0	646				1.86		4.98
Bronchial asthma	21	2	4	29.0	603	271	41.7	45.6	2.73	5.94	3.61
1.54		3	5	17.0	481	270	40.0	35.8	2.28	7.53	4.22
		4		23.0	501				1.95		4.87
G. M.	M	1		12.0	673				3.0		9.79
Sarcoidosis	26	2	4	12.5	674	268	41.3	29.0	2.8	9.23	10.8
1.91		3		22.5	656				2.77		10.9
		Anoxia—on									
		4		12.5	694				2.72		12.1
		5	8	17.5	642	294	35.2	25.8	2.83	11.4	11.1
		6	10	24.0	718	318	36.3	26.6	2.92	11.9	10.7
		Anoxia—off									
		7		20.0	724				2.80		10.3
		8	4	15.0	680	343	50.9	36.2	2.73	9.48	10.5
Cardiac patients											
A. P.	M	1		31.0	166				1.6		4.32
Cor pulmonale	42	2	3	29.0	146	286	186	85.8	1.9	3.33	3.57
1.97											
F. R.	F	1		28.5	409						5.82
R. H. D., M. S., C. H. F.‡	30	2	4	20.5	381	194	12.9	37.1	1.5	5.28	4.92
1.49		3	3	10.5	440	194	27.5	40.1	2.4	4.82	3.50
S. W.	F	1	3	37.5	169	230	32.4	84.3	2.4	2.73	4.02
R. H. D.	61	2	4	23.5	157	212	37.5	75.8	2.6	2.79	3.06
1.40		3	3	30.0	141	279	37.5	84.9	2.0	3.29	3.43
G. M.	F	1	5	20.5	144	202	22.3	96.2	9.5	2.10	1.76
R. H. D., M. S., C. H. F.	37	2	5	21.5	112	200	22.4	95.6	11.2	2.09	1.43
1.43		3		34.0	121				10.5		1.53
		Digoxin									
		4		19.0	142				8.7		1.81
		5		22.0	177				6.2		2.54
		6	5	21.0	140	203	22.6	81.5	4.05	2.49	3.90

TABLE I—*Continued*

Patient Diagnosis, B.S.A.*	Sex, age	Period	Duration O_2	Duration PACA	C_{PACA}	O_2 cons.	O_2 cons. l. ventil.	A–V diff. O_2	A–V diff. PACA	Cardiac output O_2	Cardiac output PACA†
			min.	*min.*	*ml./min.*	*ml./min.*	*ml./l.*	*ml./l.*	*mg./l.*	*l./min.*	*l./min.*
E. W. (pre-op)	F	1	3	31.0	251	212	32.8	59.2	2.7	3.58	2.89
R. H. D., M. S., C. H. F.	50	2		29.5	211				2.95		2.93
1.77		3	4	40.0	254	234	34.9	64.7	3.2	3.61	4.04
		Exercise									
		4	5	36.0	260	324	28.8	63.1	2.4	5.14	5.48
E. W. (post-op)		1	4	15.0	269	234	42.1	40.0	2.2	5.84	5.43
		2		19.5	288				1.9		5.51
		3	5	19.0	263	212	42.6	39.7	2.0	5.34	5.94
		4		17.0	282				2.15		5.70
		5		23.0	301				2.35		4.96
		Exercise									
		6	6.5	29.0	281	290	35.0	42.0	2.45	6.90	5.30
		7		17.0	311				2.43		5.22
M. H.	F	1	6.5	39.0	99	172	23.7	77.4	4.5	2.22	2.11
R. H. D., M. S., C. H. F.	30	2	6	29.5	93	160	24.2	83.0	3.6	1.92	2.01
1.34		3		23.5	90				3.4		2.65
M. Im.	F	1		19.0	297				3.3		3.52
R. H. D., M. S., C. H. F.	34	2	5	13	286	181	33.3	53.4	3.5	3.40	3.32
1.54		3		18.25	283				3.52		3.30
		Exercise									
		4		23.0	256				3.38		3.89
		5	5	13.75	223	190	34.9	52.1	3.2	3.65	4.09
F. R.	M	1		18.0	242				3.55		3.07
R. H. D., M. S., C. H. F.	41	2	5	12.0	246	218	27.9	73.7	3.75	2.96	3.34
1.72		3		22.0	257				3.7		3.68
		4	4	10.5	253	198	29.8	70.0	3.67	2.82	3.68
		5		27.0	232				3.74		3.58
		Exercise									
		6	7	29.5	114	213	26.9	61.2	3.63	3.48	3.60
		7		20.0	385				3.42		3.71
J. C.	M	1	5	25.0	493	309	33.1	55.8	1.72	5.54	5.32
R. H. D., M. S., C. H. F.	34	2		23.0	456				1.89		4.52
1.92		3	6	16.5	415	347	34.7	64.2	1.83	5.40	4.99
		4		22.0	385				1.41		4.45
		Exercise									
		5	3	12.0	253	528	30.7	81.2	1.26	6.50	5.90
		6		18.0	354				1.38		7.32
L. J.	F	1	5	12.5	488	189	40.4	46.7	2.55	4.05	4.74
R. H. D., M. S., C. H. F.	19	2		11.5	477				2.3		5.12
1.74		3	6	11.5	387	200	40.4	45.8	2.4	4.38	4.90
		4		24.0	463						
		Exercise									
		5	6	11.5	430	205	28.8	52.1	1.22	3.94	5.14
		6		9.5	242				1.8		6.54
		7		16.25	430				2.35		4.98
		Hypervent.									
		8	4.5	8.75	598	235	13.7	46.4	2.85	5.07	4.70
		9			480				2.88		4.02
		10			527						
		11			373						
D. D.	F	1		15.5	409				1.87		4.03
R. H. D., M. S., C. H. F.	30	2	10	19.0	365	196	44.7	41.1	2.01	4.77	3.42
(mild)		3	8	32.5	400	189	41.7	41.0	1.92	4.62	3.95
1.56		Exercise									
		4		9.5	497				1.35		
		5	8	13.5	425	204	39.9	43.5	1.41	4.68	5.14
		6		13.0	337				1.18		4.75

* B.S.A. = Body surface area (M^2).

† Based upon the total plasma clearance as calculated from the infusion rate and arterial plasma concentration of PACA (equation 9) and the integrated A – MV difference.

‡ R.H.D.—Rheumatic heart disease.
M.S.—Mitral stenosis.
C.H.F.—Congestive heart failure.

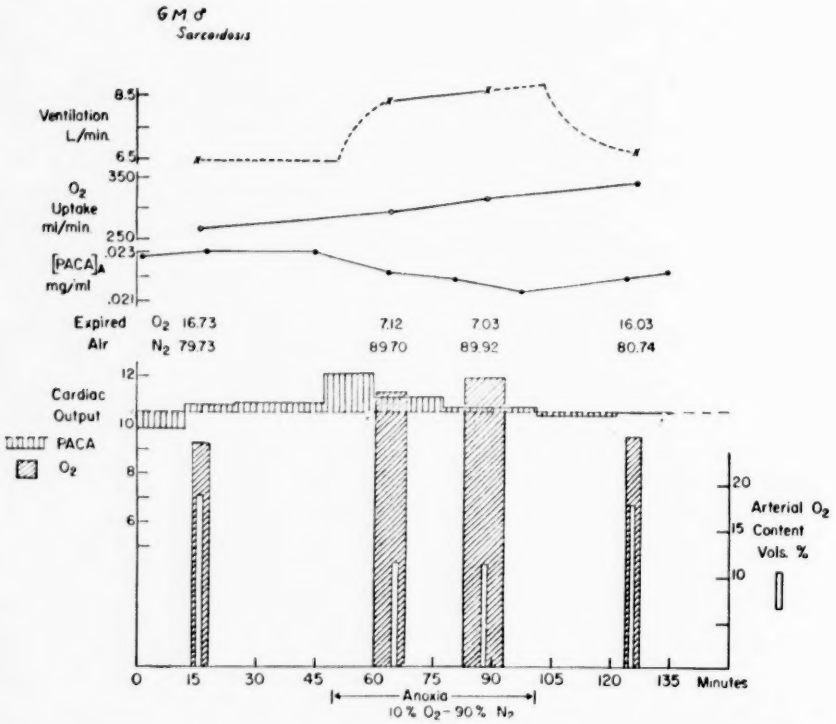

FIG. 3. EFFECT OF HYPOXEMIA ON THE CARDIAC OUTPUT AS MEASURED BY THE GAS AND CHEMICAL METHODS

PAH must occur. These data may be included in a separate report.

DISCUSSION

The evaluation of accuracy of any new method for the measurement of a physiological function usually requires comparison with some previously employed technique or method which, itself, should but may not be accurate. For example, the earlier methods of cardiac output determination involved such large error that the later, more refined measurements differed significantly (18). Despite this fact, they gained general acceptance.

Because both the chemical and gas methods utilize the Fick principle, based upon the actual withdrawal of mixed venous blood, generally good agreement between the two measurements of cardiac output, as observed in the present series, might have been anticipated. However, several factors may lead to certain differences in the results. Firstly, since the measured flow represents an average for the time of the determination, slight differences in the calculated cardiac output may result when the periods of simultaneous measurement by the two methods are not of the same duration. In order to compare the results based upon urinary excretion (equation 7) with those from the rate of infusion (equation 9), the cardiac outputs by the PACA method were arbitrarily calculated for the ten to forty minute periods of urine collection. The fact that the other cardiac outputs were based upon the usual three to six minute gas collection periods might have contributed to the observed differences. It should be emphasized, however, that outputs based upon the rate of infusion and integrated A-MV differences of the test substance require no urine collections, and may be calculated for considerably shorter periods.

Secondly, the apprehension and anxiety produced by the nose clamp and mouth piece tended to increase the cardiac output during the period of

expired gas collection above that obtained during the longer interval of urine collection.

Thirdly, since the hematocrit is employed in the calculations of the chemical method, correction for trapped plasma would have lowered the measured output by 1 to 2 per cent.

The good agreement between the results of the two methods notwithstanding the above factors supports the validity of the chemical dilution method.

For the successful application of the Fick principle, maintenance of a state of equilibrium is required if the A-V difference is to reflect accurately the change in blood flow. Because the A-MV difference is usually small, slight absolute variations in the concentration of either the arterial or mixed venous blood, due either to analytical error or to changing levels, may produce large errors in the calculated flow.

In the gas method, any variation of cardiac or respiratory function, or the content of the respired gases, will be reflected both in the measured gas utilized or produced and the A-MV difference, until a steady state, or equilibrium between the blood gas and alveolar air, has been reestablished (19). For example, with a sudden change from breathing ambient air to a 10 per cent oxygen-90 per cent nitrogen mixture, the spurious high oxygen content of the exhaled gas resulting from exhalation of oxygen previously inhaled, will produce a falsely low figure for oxygen utilization. Simultaneously, as alveolar oxygen concentration decreases, the rate of fall in oxygen concentration of arterial as compared with that of mixed venous blood also will affect the A-MV difference. To the extent that these rates differ, a spurious difference will be obtained.

Similarly, a change from room air to 100 per cent oxygen inhalation will also affect the measurement of A-MV differences and oxygen consumption. Moreover, hyperventilation, tachypnea of exercise, and other respiratory or cardiac fluctuations affecting pulmonary gas content will produce errors of varying degree in cardiac output determination until near equilibrium is attained.

By the same token, sudden changes in the cardiac output, renal blood flow or renal fraction will lead to changes in the arterial and mixed venous blood concentrations of PACA administered at a constant rate until re-equilibration occurs. Thus, during rising blood levels, the movement of PACA from plasma into interstitial fluid will produce an increased A-MV difference and, therefore, falsely low output; conversely, during falling levels, the diffusion of PACA from the extracellular fluid into the blood will yield a low A-MV difference and apparently high output. It is evident, therefore, that in the chemical adaptation of the Fick principle, as well as in the gas method, the absence of equilibrium or steady state may be a source of significant error.

However, except in the presence of markedly changing renal blood flow, the slopes of the arterial and mixed venous plasma levels are small, and the effects of PACA redistribution likewise relatively less. In such cases, more frequent withdrawal of blood specimens, the use of longer periods for measurement of cardiac output, and the application of the slope correction factor further reduce possible error.[6] Moreover, the low molecular weight and rapid diffusibility of PACA and the extensive capillary and cell membrane surface area for transfer promote rapid equilibration between blood, extracellular fluid and tissues except in very rapid changes of blood concentration.

Thus, the present method, although subject to the same errors resulting from dysequilibria, unlike the gas method, is less influenced by changes in respiratory function. Consequently, it may be of special value in recording early changes in cardiac output during respiratory changes without the delay required for pulmonary equilibration, provided that renal flows are not significantly altered. Conversely, the gas method would be preferable for studying the effect on cardiac output of drugs like epinephrine which produce a marked change in renal blood flow, thus upsetting the PACA equilibrium.

The observations on the effects of hypoxia in patient G. M. illustrate these points. Whereas the chemical method indicated an immediate rise in cardiac output with the induction of hypoxia, followed by a return to the control level, the gas

[6] This problem may be further complicated, in the case of substances like PACA, which enter certain cells. When this migration is very rapid, it has little effect on the A-V difference. Should the equilibration time across cell membranes be prolonged relative to that across the capillary membranes, longer periods must be employed to obtain true A-MV differences and accurate outputs.

method indicated a gradual rise of cardiac output throughout the period of hypoxia, falling only after return to breathing air. The reasons for the lower gas output on shifting from higher to lower oxygen breathing have been discussed above. It is of interest that earlier studies from Cournand's laboratory (20) had indicated decreased cardiac output with hypoxia. This result was re-interpreted later (19), when the implications of the steady state were better understood. In the present study, permitting more time for equilibration during the hypoxic period prior to measurement of cardiac output would have resulted in failure to demonstrate any significant change. Thus, Lewis and Gorlin (21) produced an equivalent degree of hypoxia in dogs, and after allowing time for equilibration, found no change in cardiac output.

The relatively simple operations involved in the chemical method provide a certain elasticity not present in the gas method, which ordinarily requires mask, Tissot spirometer or Douglas bag, air tank, tonometers, etc. In contrast, with the catheter *in situ* and the constant infusion running, the chemical method requires merely periodic withdrawal of arterial and mixed venous blood, since even urine collections are not necessary. This procedure is painless and can easily be accomplished under diverse conditions, although it does not obviate the need for cardiac catheterization.

The critical influence of the A-MV difference on the accuracy of any measurement by the Fick principle should be re-emphasized. Of special importance are the variations in A-MV difference with the level of cardiac output. In congestive failure, as the cardiac output falls, with either unchanged or somewhat increased oxygen consumption, the A-MV difference rises considerably. Consequently, the accuracy of the gas method is actually increased in cardiac subjects. Under similar circumstances, the chemical method employing PACA behaves quite differently. Because of the decreased renal plasma flow, excretion of PACA decreases so that any tendency for the A-MV difference to rise with falling output is counteracted. In fact, the greatly diminished renal fraction of the total blood flow reduces the A-MV difference. The PACA method, therefore, is least accurate in conditions with low cardiac output and decreased renal fraction. Conversely, where the cardiac output is high without change in oxygen consumption or renal fraction, the gas method is less and the chemical method relatively more accurate. Therefore, the present studies on a group of subjects predominantly with advanced mitral disease and congestive failure subjected the chemical method to a most severe test. That correlation remained good even under these conditions attests to the validity of the method.

A more quantitative evaluation of the two methods in terms of the A-MV difference may be made by comparing the ratios of the A-MV difference of each to the error of determination of the blood concentration.[7] Assuming a 1 per cent error in the PACA method, the quotient in normal subjects will be 15 to 18. (*i.e.*, 15 to 18%/1%); similarly, a 0.1 volume per cent error in blood gas analyses will yield a value of about 40 (4 vols.%/0.1 vol.%) and a 0.2 volume per cent error, of 20. Thus, under normal conditions, the accuracy of the gas method would be expected to be greater than that of the chemical method employing PACA.

However, further improvement of the chemical method may yet be attained. For example, the use of a different test substance with large general tissue uptake not limited to the kidneys would result in much higher A-MV differences. It is essential that the rate of uptake be fairly constant, the analysis simple and accurate, and equilibrium rapidly attained. Several substances including fructose and some isotopically labelled compounds are being considered.

In a sense, then, the results herein reported, while supporting the inherent validity of the chemical method, are preliminary. Further refinement and greater accuracy would appear to be readily capable of achievement.

SUMMARY

1. A method in which the direct Fick principle for the measurement of cardiac output is modified for the use of a non-gaseous test substance is described.

[7] The fraction, A-MV difference/analytic error, is simply an index of the accuracy of the method. In practice, in each method, since two analyses determine the A-MV difference, the actual error is doubled. The fraction employed is simply for comparison.

2. In a series of 18 patients, 11 of whom had heart disease with congestive failure of varying severity, this method was employed together with the usual oxygen method and good correlation ($r = 0.90$) was obtained. In one patient, the effect of moderate hypoxemia on the determination by both methods was studied.

3. The relative merits of the two methods under conditions of respiratory dysequilibria, varying cardiac output and renal fraction are discussed.

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HEXAMETHONIUM—ITS EFFECT ON GLOMERULAR FILTRATION RATE, MAXIMAL TUBULAR FUNCTION, AND RENAL EXCRETION OF ELECTROLYTES[1]

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Hexamethonium chloride is an autonomic ganglionic blocking drug which has been reported to be effective in reducing blood pressure (1–3). In patients with severe hypertensive vascular disease the brain and the heart appear to be benefited by a moderate reduction in blood pressure (2–5). However, since glomerular filtration is dependent upon systemic blood pressure, one might anticipate that renal excretory capacity may be affected adversely by this procedure, particularly if kidney disease is associated with the hypertension. For this reason, the following study was undertaken, in which renal functions were evaluated before and after reduction in blood pressure with hexamethonium.[2] The studies were carried out on control subjects with normal blood pressure and on patients with hypertension with and without renal damage as estimated by glomerular filtration rate.

METHODS

Observations on the effect of hexamethonium administered intravenously were made on 14 control subjects (six men and eight women) with normal blood pressure and on 22 patients (13 men and nine women) with hypertension. Maximal tubular function (TmPAH) before and after blood pressure reduction was determined on four of the normal subjects and on six of the patients with hypertension. Para-aminohippurate (PAH) at plasma levels of 60 to 90 mg. per 100 ml. of plasma was used for this measurement. The analytical method of Smith and his associates (6) was used for the determination of PAH. The effect of blood pressure reduction on glomerular filtration rate (GFR) and on water and electrolyte excretion was determined on ten of the normal subjects (Group A, Table II); on five of the patients (Group B, Table II) with hypertension but relatively normal kidney function as determined by a GFR[3] of more than 80 ml. per minute; and on 11 of the patients (Group C, Table II) with hypertension and moderate to marked renal damage as determined by GFR[3] less than 80 ml. per minute. Glomerular filtration (GFR) was determined in all patients by the inulin clearance method at plasma levels of 30 to 60 mg. per 100 ml. of plasma. Roe's analytical technique was used (7). Plasma sodium and potassium concentrations and the amounts of these electrolytes excreted in the urine were determined using a Beckman flame photometer for analysis. Electrolyte excretion was not determined on the patients on whom TmPAH studies were done because of the diuretic effect of PAH and because this agent increases urinary sodium excretion, thus obscuring any effect due to the hexamethonium.

The blood pressure was determined by the auscultatory method as well as by intra-arterial manometry. In a few patients on whom auscultatory observations alone were made, the mean blood pressure was calculated by the diastolic blood pressure plus one-third of the pulse pressure. The intra-arterial needle was connected to a mercury manometer through a manifold which was also used for collecting blood samples. Blood for analysis was collected two minutes prior to the midpoint of each 10-minute collection period. A mercury pump (8) was used for maintaining a constant intravenous infusion of inulin and PAH. Rising or unchanging blood levels of inulin and PAH were used. Experiments showing falling blood levels were discarded. Urine samples were obtained through an indwelling catheter using air and 100 ml. of distilled water as a bladder wash. Following the priming doses of PAH and inulin, a 30-minute equilibration period was allowed before the experiment was started. Control observations consisted of three or four consecutive 10-minute periods. Following the control studies, hexamethonium was administered slowly by the intravenous route over a 10- to 20-minute period. The dose of hexamethonium was determined largely by the blood pressure response to the drug. In the hypertensive patients, an attempt was made to reduce the mean blood pressure 20 to 30 mm. of Hg or more, unless the patient experienced uncomfortable side reactions. The mean blood pressure was reduced approximately 10 to 20 mm. of Hg in the normal control subjects. Renal function studies (10-minute periods) were carried out during the

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[3] Corrected to standard body area (1.73 sq. m.).

drug administration and for one to two hours thereafter. Five patients were studied for three hours.

In addition to the previous studies, the effect of norepinephrine infusion after hexamethonium was determined on nine patients. Norepinephrine was given to two of these patients as a therapeutic measure. The study was conducted on the remaining seven only for purposes of evaluating renal hemodynamic response. In this phase of the study, the control renal function observations were carried out as outlined above. When maximum hypotension was established and the blood pressure stabilized for 30 minutes (Patients Nos. 1 and 2 excepted) or more, norepinephrine was administered by continuous infusion using a concentration of 4 micrograms per ml. of 5 per cent glucose. The height of the blood pressure was regulated by the rate of infusion. Renal function studies were then repeated using the same technique as previously outlined.

RESULTS

The blood pressure response to the intravenous administration of hexamethonium in one of the patients is seen in Figure 1. Within ten minutes there was a sharp reduction in blood pressure in most patients, followed by a slight rise and then stabilization at a point somewhat above the lowest level attained. In a few patients the blood pressure decreased slowly and progressively over a 10- to 20-minute period. A reduction in blood pressure was obtained in all patients included in the present study. However, three additional patients who received the drug were not included because there were no significant alterations in blood pressure or in renal function. The percentile reduction in systolic and diastolic pressures were about equal with a resultant decrease in pulse pressure. There was no significant increase in pulse rate. The blood pressure in most of the patients remained at this reduced level for one hour or more and then slowly rose so that after three to four hours it was back to or approaching the control level. However, in some patients, a significant reduction in blood pressure persisted for eight to 12 hours (Figure 1). The effects of hexamethonium on blood pressure and pulse rate are summarized in Table I. The average percentile reduction in blood pressure was about as great in the patients with normal blood pressure as in the hypertensive patients. However, in absolute terms the decrease was twice as great in the patients with hypertension. The average patient without renal disease (Group B) required less hexamethonium than the average patient with renal disease (Group C), but more of the patients with severe and malignant hypertensive vascular disease fell into the latter group. The more rapid the rate of hexa-

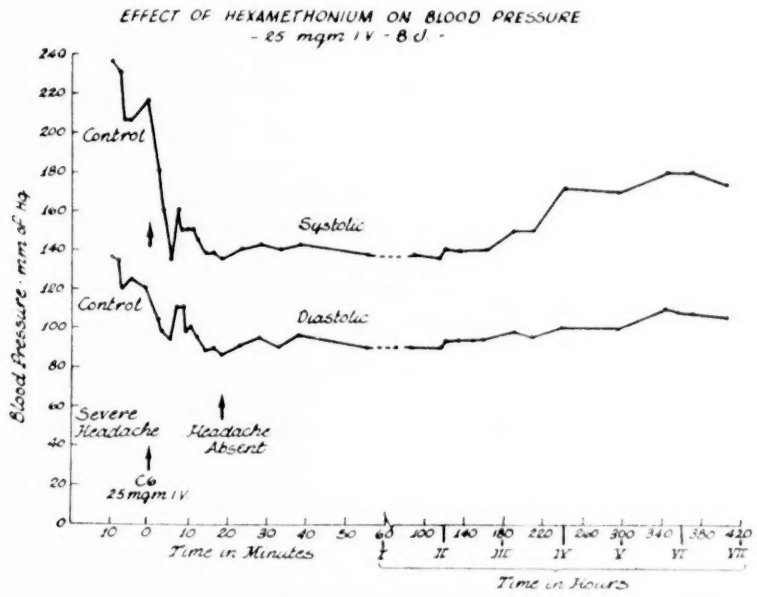


FIG. 1. TYPICAL BLOOD PRESSURE RESPONSE TO INTRAVENOUS HEXAMETHONIUM

TABLE I

Effect of hexamethonium on blood pressure and pulse rate

	MBP*				PR†				
	C	D_1	D_2	D_3	C	D_1	D_2	D_3	Dose‡
Group A. Control subjects with normal blood pressures									
Ph.	93	87	69	85	84	90	84	—	25
Si.	89	77	77	81	106	120	118	102	25
Al.	97	82	75	88	84	80	84	—	15
Co.	81	75	68	73	68	80	76	76	25
Jo.	84	78	71	77	—	—	—	—	50
Jn.	84	70	77	77	68	76	80	74	10
Ca.	105	83	86	102	92	84	82	84	25
Sc.	105	95	99	96	84	90	84	78	30
Ra.	78	66	71	74	92	88	84	80	25
Jd.	94	77	76	77	90	78	76	80	25
Mean	91	79	77	83	85	87	85	82	26
% of Control		87	85	91		102	100	96	—
Group B. Hypertensive patients with minimal renal change, i.e., GFR more than 80 ml./min.									
Jo.	139	125	105	100	—	—	—	—	20
Wa.	169	123	122	125	88	88	88	88	20
Ha.	141	120	110	121	92	104	100	104	10
Ro.	143	100	97	98	—	—	—	—	25
Mo.	147	137	119	123	80	76	80	84	10
Mean	148	121	111	113	87	89	89	92	17
% of Control		82	75	76		102	102	106	—
Group C. Hypertensive patients with renal damage, i.e., GFR less than 80 ml./min.									
Cr.	170	132	134	142	—	—	—	—	5
Ca.	143	120	98	98	—	—	—	—	25
Ha.	191	157	174	184	—	—	—	—	25
St.	166	130	122	122	—	—	—	—	45
Wo.	146	129	130	135	—	—	—	—	60
Ha.	153	104	111	133	88	88	80	80	20
Nu.	151	136	129	130	—	—	—	—	50
Mc.	156	147	145	143	88	88	84	84	20
Hr.	146	114	103	124	84	92	84	92	40
Pe.	176	166	157	160	72	28	80	76	75
Sa.	129	86	81	88	92	88	94	84	25
Mean	157	129	126	133	85	77	84	83	35
% of Control		82	80	85		91	99	98	—

* MBP—Mean blood pressure, diastolic plus one-third of the pulse pressure.
† PR—Pulse rate.
‡ Dose—milligrams given parenterally.
C—Control.
D_1—During intravenous infusion of hexamethonium, 10- to 20-minute period.
D_2—Average of two periods during maximum reduction in blood pressure after completion of D_1.
D_3—One hour after hexamethonium infusion.

methonium administration the more precipitous was the reduction in blood pressure. It was a common observation that initially the infusion of the drug caused a progressive reduction in blood pressure until a floor in the blood pressure was reached. At this point, maximal blockade for this drug was apparently approached and considerably greater amounts of hexamethonium could be given without bringing about much more of a reduction in blood pressure. Usually there was no great difference in the mean blood pressure during the drug infusion (D_1) (after initial reduction), at the point of maximum depression (D_2), and one hour after giving hexamethonium (D_3) both in the normotensive and the hypertensive patients. In several instances the mean blood pressure was lower during the period of drug infusion (D_1) than at any time thereafter (D_2).

Associated with the blood pressure reduction, there was a sharp decrease in GFR (Table II)

during the period of 10 to 20 minutes while the hexamethonium was being administered (period D_1). When compared with the mean of the control observations, the mean value for GFR during this period (D_1) showed a greater percentile reduction in the patients with hypertension (Groups B and C) than in the normals (Group A), although the percentile reduction in mean blood pressure was about equal. However, following the drug, the absolute blood pressure was con-

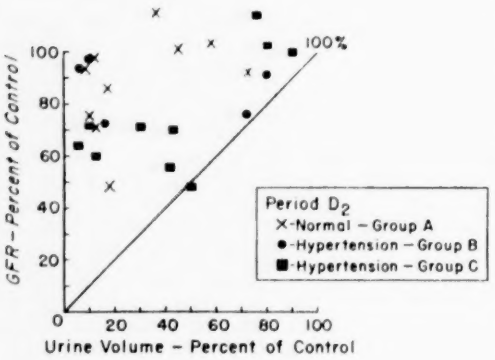
TABLE II

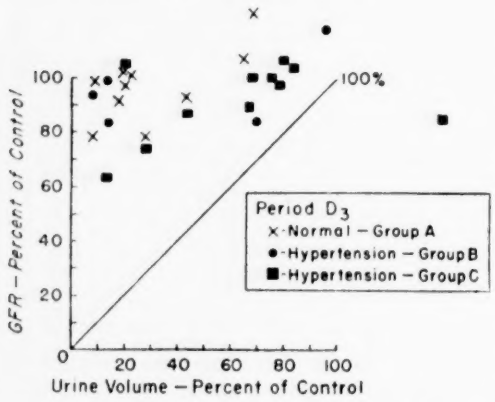
Effect of blood pressure reduction with hexamethonium on glomerular filtration rate and on the rate of excretion of sodium, potassium, and water by the kidney

Pt.	GFR* C	GFR* D_1	GFR* D_2	GFR* D_3	UV† C	UV† D_1	UV† D_2	UV† D_3	Na Excr.‡ C	Na Excr.‡ D_1	Na Excr.‡ D_2	Na Excr.‡ D_3	K Excr.§ C	K Excr.§ D_1	K Excr.§ D_2	K Excr.§ D_3	U/P In.¶ C	U/P In.¶ D_1	U/P In.¶ D_2	U/P In.¶ D_3
Group A. Control patients with normal blood pressure																				
Ph.	87	96	41	—	1.1	1.5	0.2	0.3	3.67	4.02	0.74	—	2.12	2.00	1.16	—	84	80	289	—
Si.	114	84	85	89	7.5	1.7	0.7	0.6	5.87	3.52	3.21	4.68	1.78	1.45	1.65	1.85	17	69	163	170
Al.	120	88	85	116	8.5	1.1	1.0	1.7	2.46	1.02	1.11	1.76	2.33	1.80	3.39	—	10	29	34	—
Co.	121	125	104	110	4.8	4.9	0.8	0.9	5.22	4.31	1.43	—	3.37	2.91	3.18	3.76	25	27	136	121
Jo.	121	128	113	112	8.3	9.1	6.0	3.6	8.68	7.23	5.25	6.54	3.29	3.11	2.86	3.33	15	14	20	32
Jn.	104	105	108	106	14.4	11.8	8.3	3.2	3.38	1.32	1.20	2.34	3.62	3.40	3.20	—	9	11	16	—
Ca.	109	45	125	135	2.5	0.6	0.9	1.7	3.88	1.48	0.76	1.40	3.46	1.38	5.53	5.50	50	75	152	79
Sc.	103	83	101	105	5.7	1.1	0.6	1.2	4.45	2.00	3.20	3.40	4.40	5.70	5.55	5.64	18	73	162	—
Ra.	163	166	167	175	15.0	7.2	6.7	9.7	4.62	1.98	2.04	3.58	1.96	2.06	3.12	3.29	11	25	26	18
Jd.	140	126	132	137	19.2	4.2	1.8	1.8	3.88	1.61	1.94	2.50	2.75	2.38	2.48	2.49	8	27	77	76
Mean	118	105	106	121‖	8.7	4.3	2.7	2.5	4.61	2.85	2.09	3.28‖	2.91	2.62	3.21	3.69‖	25	43	108	83‖
% of Control		89	90	103		49	31	29		62	45	71		90	110	127		172	432	332
Plasma Sodium (mEq.) same periods (mean—10 pts.)									146	148	147	148								
Group B. Hypertensive patients with minimal renal damage, i.e., GFR more than 80 ml./min.																				
Jo.	140	69	136	138	10.1	3.7	1.0	1.3	2.82	3.00	0.85	0.98	4.36	3.46	4.02	4.78	13	23	132	94
Wa.	81	28	62	68	16.8	12.5	12.3	11.8	6.28	3.65	4.74	4.60	1.25	1.33	1.45	1.60	5	2	5	6
Ha.	85	84	77	100	11.4	9.3	9.1	11.0	5.28	3.03	3.73	4.00	1.31	1.36	1.26	1.42	7	9	8	10
Ro.	140	38	131	130	9.9	1.1	0.9	0.8	7.47	1.50	1.23	0.93	2.72	4.35	3.26	3.38	16	42	145	180
Mo.	103	64	74	86	4.3	0.8	0.7	0.6	15.61	7.92	6.16	5.75	6.39	2.30	3.62	6.90	26	90	120	162
Mean	110	57	96	104	10.5	5.5	4.8	5.1	7.49	3.82	3.34	3.25	3.21	2.56	2.72	3.62	13	33	82	90
% of Control		52	87	95		52	46	49		51	45	43		80	85	113		254	631	692
Plasma Sodium (mEq.) same periods (mean—5 pts.)									147	150	148	147								
Group C. Hypertensive patients with renal damage, i.e., GFR less than 80 ml./min.																				
Cr.	67	45	69	60	1.2	0.8	1.0	0.8	1.42	0.78	1.00	1.29	2.11	1.45	2.98	2.30	52	53	64	66
Ca.	21	24	12	22	1.0	0.9	0.4	0.8	1.10	1.55	0.22	0.25	2.42	2.78	1.35	1.20	23	17	19	24
Ha.	33	17	23	33	7.4	2.0	3.2	5.8	7.15	1.54	2.22	4.78	1.74	0.92	1.20	1.24	4	8	7	5
St.	58	34	35	37	8.6	0.7	1.0	1.1	6.80	1.52	0.60	0.64	2.18	1.20	0.66	0.64	8	59	38	36
Wo.	24	16	17	18	5.4	1.5	1.6	1.5	2.84	0.78	1.46	1.42	1.59	1.56	1.72	1.64	5	12	10	12
Ha.	38	20	28	39	6.0	0.2	0.6	1.2	3.18	0.35	0.60	1.66	1.66	1.25	1.58	1.91	6	95	43	28
Nu.	8	7	8	8	2.2	2.1	2.0	1.5	1.99	1.89	1.97	1.60	1.71	1.68	1.88	1.84	4	3	5	6
Mc.	7	7	8	7	2.0	0.8	1.5	1.5	4.40	3.52	4.00	4.64	1.15	0.97	1.21	1.42	4	8	5	4
Hr.	66	36	42	59	6.6	1.3	0.3	2.9	3.08	1.10	2.00	2.47	3.43	3.50	3.70	3.60	10	25	130	—
Pe.	34	37	26	37	7.4	7.4	—	5.9	2.68	3.99	3.43	3.89	2.07	2.59	2.94	2.91	24	6	—	8
Sa.	39	18	19	33	0.6	0.4	0.3	1.0	0.34	0.19	0.19	0.54	3.30	1.90	1.95	3.80	58	40	65	24
Mean	36	24	26	32	4.4	1.6	1.2	2.2	3.18	1.56	1.61	2.11	2.12	1.80	1.92	2.05	18	30	39	21
% of Control		67	72	89		36	27	50		49	51	66		85	91	97		167	217	117
Plasma Sodium (mEq.) same periods (mean—11 pts.)									142	141	142	144								

* GFR—Glomerular filtration rate, ml./minute—corrected to standard body area.
† UV—Urine volume, ml./minute.
‡ Na excr.—Sodium excretion, mg./minute.
§ K excr.—Potassium excretion, mg./minute.
¶ U/P in.—Urine concentration Inulin/Plasma concentration Inulin.
‖ Average control values for patients in D_3 series: GFR = 122; Na excr. = 4.65 mg.; K excr. = 3.00 mg.; and U/P inulin = 21.
C—Control.
D_1—During intravenous infusion of hexamethonium, 10- to 20-minute period.
D_2—Average of two periods during maximum reduction in blood pressure after completion of D_1.
D_3—One hour after hexamethonium infusion (average values for 2 periods).

siderably lower in the normal individuals (average MBP = 79 mm. Hg) than in the two groups of hypertensive patients (average MBP = 121 and 129 mm. Hg). The observations on renal function following the period of drug infusion and after maximum hypotension was attained are presented under column D_2, Table II. These values represent the average of two successive 10-minute periods. The one-hour observations (D_3) also represent the average of two successive 10-minute periods. Following the initial acute reduction in glomerular filtration rate (GFR), which was most marked during the period of drug administration, there was a gradual return toward control levels. After one hour, GFR approximated the control observations in the majority of both normotensive and hypertensive patients. In this respect, there was a difference in the hypertensive patients with renal damage and those without. At the point of maximum reduction in blood pressure (D_2) both the normotensive and hypertensive patients with normal renal function apparently adapted to the reduced pressure more rapidly than those with renal damage. As a result, the average GFR for the normal group was 90 per cent of the control; the average GFR for the hypertensive group with normal kidneys (Group B) was 87 per cent of the control; but for the patients with renal damage it was 72 per cent of the average of the control observations. This difference in renal readjustment to the reduced blood pressure could also be noted after one hour. The mean value for GFR in the normal group was 103 per cent of the control, while in the hypertensive patients with renal damage, it was only 89 per cent. The differences in adaption to lowered pressure were greater in some patients than in others since the number of patients in whom GFR failed to return to absolute control levels was about equivalent in both groups. This is of significance in that the excretory capacity as reflected in blood urea nitrogen was already impaired in many of the patients in the latter group. Therefore, only a small reduction in GFR could be expected to depress further (at least temporarily) renal excretory function in contrast to the wide margin of renal excretory reserve which exists in patients with relatively normal kidneys.



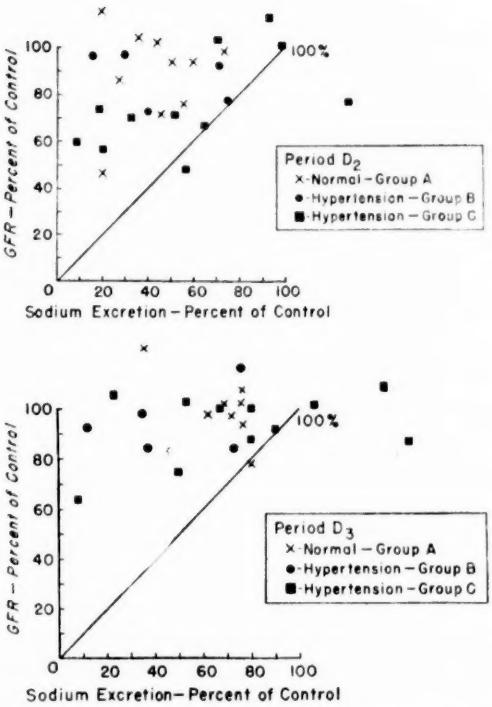


FIGS. 2A AND 2B. GLOMERULAR FILTRATION RATE IS PLOTTED AGAINST URINE VOLUME. THE VALUES ARE EXPRESSED IN PER CENT OF THE CONTROL OBSERVATIONS

Figure 2A represents the observations during maximum reduction in blood pressure (D_2).

Figure 2B represents the observations one hour after the administration of hexamethonium. Urine is reduced more than glomerular filtration rate during both periods of observation. There is essentially no difference between the normal patients and those with hypertensive vascular disease.

The reduction in blood pressure following hexamethonium was associated with a marked antidiuretic effect whether or not glomerular filtration rate (GFR) was reduced. Although GFR was reduced in the majority of patients, renal tubular reabsorption of water was increased out of proportion. This is clearly demonstrated in Figures 2A and 2B in which the GFR for periods D_2 and D_3 is expressed as per cent of control and is plotted against urine volume expressed in the same manner. During period D_2 all the values for GFR except two fell above the line of unity when compared with urine volume, and these two were hypertensive patients in whom GFR and urine volume decreased proportionally. There were

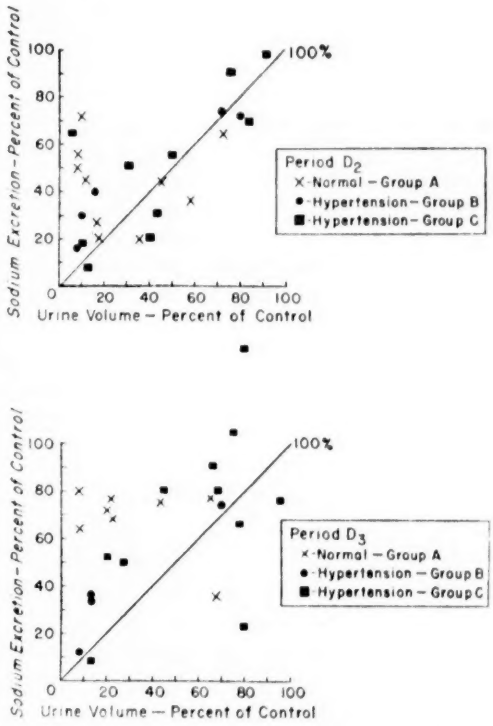


FIGS. 3A AND 3B. URINARY EXCRETION OF SODIUM IS PLOTTED AGAINST GLOMERULAR FILTRATION RATE. THE VALUES ARE EXPRESSED IN PER CENT OF THE CONTROL OBSERVATIONS

Figure 3A includes the observations during maximum reduction in blood pressure and Figure 3B those one hour after the administration of the hexamethonium. Sodium reabsorption is increased out of proportion to the reduction in glomerular filtration rate. One hour after hexamethonium, the glomerular filtration rates are returning toward the control values more rapidly than sodium excretion rates.

no obvious differences between the normotensive and the hypertensive patients and those with and without renal disease. After one hour (D_3) GFR approached or returned to the control values, but the urine volumes remained depressed to about the same degree as during period D_2. There was one exception (Sa., Table II) who was a patient with marked renal damage. In this instance, urine volume after one hour was greater than during the control period. The antidiuretic effect of acute reduction in blood pressure after hexamethonium is also reflected in the increasing U/P ratio of inulin. This was less pronounced in the patients with reduced filtration rate than in the other two groups.

Associated with the antidiuretic effect following hexamethonium, the renal tubules reabsorbed a greater proportion of the filtered sodium, resulting in a decreased excretion of this electrolyte in the urine. This also occurred independently of glomerular filtration rate, which can be visualized in Figures 3A and 3B. Here GFR for each patient is plotted against sodium excretion. Both are expressed in per cent of the control observations. During period D_2 all of the points for GFR but one fell on or above the line of unity when compared with sodium excretion. The exception again was a patient (Pe., Table II) with hypertension and renal damage. After one hour, the points continued



FIGS. 4A AND 4B. URINE VOLUME IS PLOTTED AGAINST SODIUM EXCRETION. THE VALUES ARE EXPRESSED IN PER CENT OF THE CONTROL OBSERVATIONS

During the period of maximum reduction in blood pressure (Figure 4A), urine volume and sodium excretion are decreased proportionally in most patients. This is not true in those patients showing an extreme reduction in urine volume.

to fall above the line of unity (Figure 3B), although there was some increase in sodium excretion. There were two exceptions (Sa. and Pe.), both patients with renal damage. Otherwise, it appeared that the normotensive patients and the hypertensive ones with and without renal damage responded in a similar manner. As a rule, impaired renal function as estimated by reduced GFR did not alter this effect. This response was maximal during the greatest depression in blood pressure (D_2) showing a reduction in sodium excretion of 50 per cent or more for the group.

The relationship between the reduction in urine volume and sodium excretion is graphed in Figures 4A and 4B. During the period of maximum reduction in blood pressure, sodium excretion was reduced in proportion to urine volume unless urine volume was reduced 70 to 80 per cent or more of the control observation. Above this range the points fell on or near the line of unity. Below 70 to 80 per cent the points fell on or above this line, indicating a greater reduction in water excretion than in sodium. This may represent a factor of limitation as far as sodium reabsorption is concerned but might also reflect errors in collection due to the low urine volume. After one hour (D_3) there was much less pattern to the graph (Figure 4B).

The excretion of potassium was depressed in some patients following hexamethonium but this was inconstant. There were no significant alterations in plasma sodium (Table II) and potassium during this study. Observations on five patients two to three hours after hexamethonium administration showed that the renal clearance studies all returned to control values before the blood pressure did.

When the effect of acute blood pressure reduction on maximum tubular function was determined (Table III), the glomerular filtration rate was reduced in essentially the same manner as during the previous studies (Table II). However, maximum tubular excretory capacity of PAH was not altered significantly. This is reflected in a decrease in the GFR/TmPAH ratio. It indicates that the number of functioning nephrons did not change as the blood pressure decreased, either in the normotensive group or in the patients with hypertension. The reduction in GFR resulted from decreased filtration in the active glomeruli. Kidneys showing impaired renal function as defined in this study responded in the same way as in the normal.

The response to norepinephrine after hexamethonium was evaluated in nine patients (Table IV), to two of whom it was given as a therapeutic

TABLE III

Effect of acute blood pressure reduction with hexamethonium on glomerular filtration rate and maximum tubular function

	MBP*				GFR*				UV*				TmPAH†				GFR/TmPAH			
Pt.	C	D_1	D_2	D_3	C	D_1	D_2	D_3	C	D_1	D_2	D_3	C	D_1	D_2	D_3	C	D_1	D_2	D_3
Patients with hypertension																				
Ta.	108	84	76	88	57	38	47	52	2.9	1.9	2.1	2.1	24	25	27	20	2.37	1.52	1.74	2.60
Jo.	126	106	104	113	55	50	47	51	2.7	2.5	2.7	3.0	28	26	25	30	1.96	1.92	1.88	1.70
Ph.	123	86	76	76	126	91	103	110	7.5	3.9	2.8	3.1	87	74	88	83	1.45	1.23	1.17	1.33
Ma.	178	126	107	112	69	60	48	50	3.0	2.6	2.7	2.6	52	52	58	51	1.33	1.15	0.83	0.98
Cl.	135	109	99	100	41	33	36	40	10.8	7.5	4.4	4.6	27	24	25	32	1.52	1.37	1.44	1.25
Ct.	110	60	65	70	28	16	19	20	4.6	1.9	2.3	2.4	21	20	28	28	1.34	0.80	0.68	0.63
Mean	130	95	88	93	63	48	50	54	5.3	3.4	2.8	3.0	40	37	42	41	1.66	1.33	1.29	1.42
% of Control		73	68	72		76	79	86		64	53	57		93	105	103		80	78	86
Normotensive patients																				
Fi.	94	64	70	90	125	121	129	110	6.1	3.6	3.9	5.0	89	88	93	92	1.40	1.38	1.39	1.20
Fl.	104	93	96	97	108	86	91	105	3.0	3.0	3.0	3.0	76	69	66	77	1.42	1.25	1.38	1.37
Ha.	105	100	99	100	146	132	139	135	6.1	4.1	3.5	3.6	75	78	80	81	1.95	1.69	1.74	1.67
Vi.	82	67	80	80	80	60	74	74	3.2	2.9	3.6	2.3	78	75	80	81	1.03	0.80	0.93	0.92
Mean	96	81	86	92	115	100	108	106	4.6	3.4	3.5	3.5	80	78	80	83	1.45	1.28	1.36	1.29
% of Control		84	90	96		87	94	92		74	76	76		98	100	104		88	94	89

* See Tables I and II for key to abbreviations.
† TmPAH—Maximum tubular excretion of p-aminohippurate, mg./min.

TABLE IV

Renal response to hexamethonium followed by an intravenous infusion of norepinephrine

Pt. No.	MBP* C	MBP* D_H	MBP* D_N	GFR* C	GFR* D_H	GFR* D_N	UV* C	UV* D_H	UV* D_N	TmPAH* C	TmPAH* D_H	TmPAH* D_N
1†	140	63	115	100	0?	82	1.0	0	3.4	66	—	59
2‡	165	95	149	67	13	45	0.9	1.0	0.6	—	—	—
3	173	98	153	23	25	19	5.3	2.4	3.2	—	—	—
4	123	76	140	126	110	112	7.5	3.1	4.2	87	86	83
5	129	103	184	39	30	41	0.6	0.5	3.8	21	28	30
6	139	107	161	140	123	129	—	—	—	—	—	—
7	170	123	174	67	62	57	—	—	—	—	—	—
8	107	68	119	21	22	16	1.0	1.0	0.7	—	—	—
9	180	120	184	75	67	65	3.8	2.8	2.4	57	42	42
Mean	144	95	155	73	50	63	2.9	1.5	2.6	58	52	54

C—Control.

D_H—30 to 60 minutes following hexamethonium given intravenously.

D_N—Norepinephrine infusion following previous blood pressure reduction with hexamethonium.

* See Tables I, II, and III for key to abbreviations.

† This patient developed acute hypotension following oral hexamethonium. Norepinephrine was administered therapeutically.

‡ Patient developed mental confusion and became disoriented following hexamethonium. This improved when the blood pressure was increased with norepinephrine infusion.

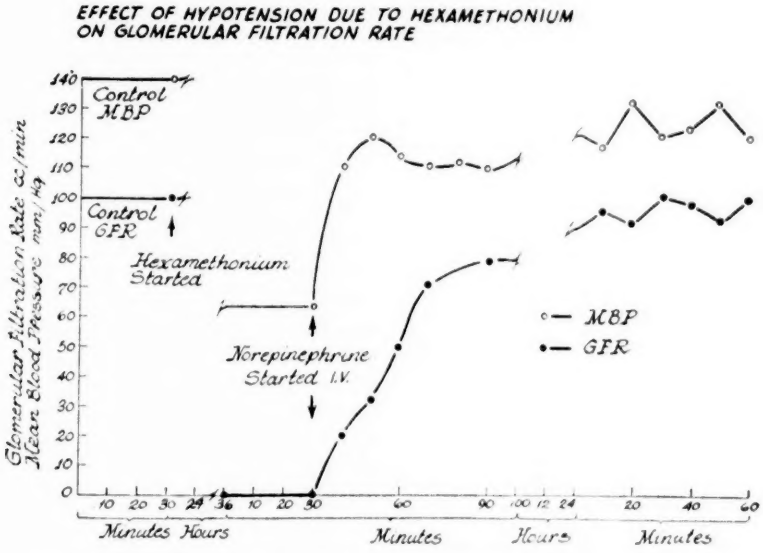


FIG. 5A. FOLLOWING AN OVERDOSE OF HEXAMETHONIUM BY THE ORAL ROUTE, THE SUPINE BLOOD PRESSURE DECREASED FROM 180 MM. HG SYSTOLIC AND 120 MM. HG DIASTOLIC TO 85 SYSTOLIC AND 52 DIASTOLIC

It remained at approximately this level for 2 days and the patient became anuric on the second day. A norepinephrine infusion was started and the blood pressure was increased to 145 systolic and 100 diastolic. Within 15 minutes the patient began to form urine. The glomerular filtration rate gradually increased until after one hour it was approaching the control level.

measure. One of the patients (Patient No. 1, Table IV and Figure 5A) had previously taken an excessive dose of hexamethonium orally, following which the mean blood pressure decreased from 140 to 63 mm. Hg and she became anuric. Renal function was measured during the control period and after the blood pressure was raised with an infusion of norepinephrine. Following an increase in the mean blood pressure to approximately 115 mm. Hg, the GFR and urinary output increased progressively; the hexamethonium was excreted and recovery was uneventful.

In the second patient (Patient No. 2, Table IV and Figure 5B), the mean blood pressure decreased from 165 to 95 mm. Hg following which the patient became mentally confused and disoriented. Glomerular filtration was depressed from 67 to 13 ml. per minute. Norepinephrine was started and the blood pressure increased. Cerebral function returned to the control state and at the same time the GFR increased to 45 ml. per minute (Figure 5B). However, when norepinephrine was discontinued after 20 minutes, the blood pressure and GFR were again depressed. After a second administration of norepinephrine for 20 minutes the blood pressure became better stabilized.

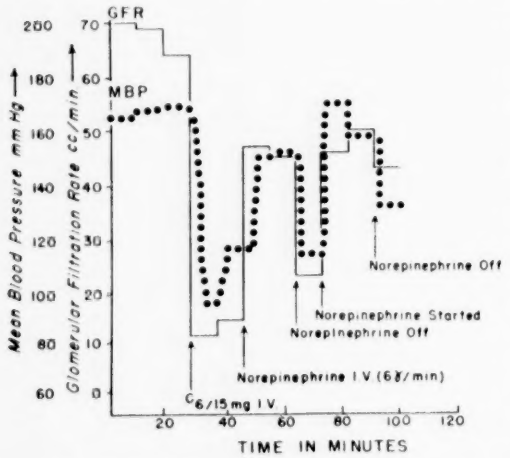


FIG. 5B. THIS PATIENT EXHIBITED SOME ENCEPHALOPATHIC MANIFESTATIONS DURING THE CONTROL PERIOD

Following hexamethonium the mean blood pressure decreased from 165 to 95 mm. Hg and the patient became disoriented. The GFR was markedly depressed (GFR of 67 decreased to 13 ml. per minute). The mean blood pressure was increased with norepinephrine to 149 mm. Hg, whereupon the cerebral manifestations and GFR improved.

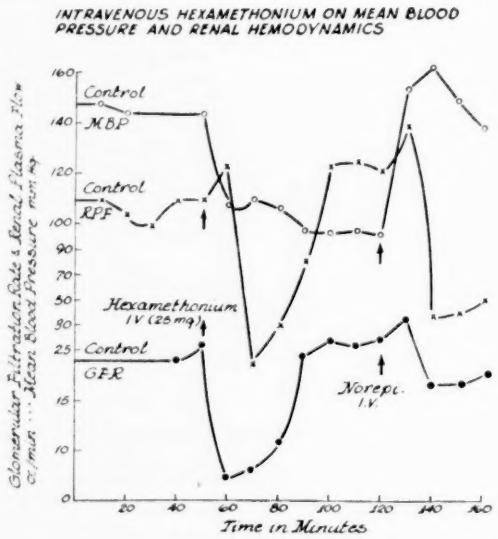


FIG. 5C. THE RENAL HEMODYNAMIC RESPONSE TO PARENTERAL HEXAMETHONIUM IN A PATIENT WITH RENAL DAMAGE (GFR = 23)

There was an acute reduction in blood pressure associated with depression of GFR. The latter gradually returned to the control level despite a maintained reduction in blood pressure. When the blood pressure was increased to the control values with norepinephrine, GFR was slightly depressed.

Norepinephrine was given to seven additional patients in order to evaluate better the renal response under these circumstances. The effect on GFR is variable and appears to be dependent on the degree of depression following the administration of hexamethonium. In general when GFR is markedly depressed by hexamethonium, norepinephrine tends to increase it toward the control values at the same time that the blood pressure is increased (Figures 5A–5B). However, in those patients in whom GFR was not markedly depressed (Figure 5C) from the control period, raising the blood pressure with norepinephrine seemed to have little effect on GFR, or actually reduced it (Figure 5C). In three patients thus studied, TmPAH was not affected significantly by this procedure. Raising the blood pressure with norepinephrine had very little effect on the antidiuretic effect of hexamethonium although sodium excretion was enhanced.

DISCUSSION

The present observations indicate that ganglionic blockade with hexamethonium was a dependable method for reducing the blood pressure in both normotensive and hypertensive individuals. As the blood pressure decreased, there was no associated reflex tachycardia, probably a result of the ganglionic blockade of the sympathetics to the heart. With some exceptions, the marked reduction in blood pressure in patients exhibiting hypertension again emphasizes the importance of the autonomic nervous system in this malady. Associated renal disease did not affect this response, suggesting that even when the kidneys are involved, imbalance of the autonomic nervous system usually plays a significant role in the development of the elevated blood pressure.

The observation of acute depression of glomerular filtration rate (GFR) following ganglionic blockade and blood pressure reduction with hexamethonium is in contrast to similar studies on anesthetized dogs (9). In the latter studies, the initial depression of GFR was not seen, indicating more rapid readjustment of renal hemodynamics in dogs. Likewise, it appears that GFR in patients with normal kidneys tended to readjust to changes in blood pressure more rapidly than it did in patients with damaged ones. However, one hour after the hexamethonium was given, these differences were not so apparent, and very little depression of GFR was noted despite the maintained reduction in blood pressure. This response of patients to hexamethonium is qualitatively similar to autonomic blockade with tetraethylammonium (10). After the latter drug there is also an initial depression of renal function followed by readjustment to a lower blood pressure. Subsequently, renal functions gradually return to control levels.

The renal dynamics in patients with hypertension respond to hexamethonium in essentially the same way as do those in patients with normal blood pressure. Patients with evidence of moderate to severe renal disease react qualitatively in a similar manner. This indicates that the nephrons that remain functional apparently react in a normal fashion and supports the contention that in the damaged kidney nephrons are destroyed as anatomical and functional units. Those nephrons that remain, function in a relatively normal fashion. There is no evidence to indicate why glomerular filtration is reduced and remains depressed in some patients more than others despite equivalent absolute blood pressure reductions in both.

In patients with relatively normal kidneys, as long as the blood pressure is not reduced below critical filtration levels, renal excretory capacity will be adequate, even though GFR may be somewhat depressed. However, when renal excretory capacity and glomerular filtration are already impaired due to anatomical changes in the kidneys, then only slight reductions in GFR may further jeopardize this excretory capacity and thus decompensate the renal mechanism. There seems to be no way to predict which patients will not readjust their renal hemodynamics and filtrating capacity to a lower blood pressure, since some of the patients with the most renal damage readjusted as completely as the normotensive ones, although the former was at a higher absolute level of blood pressure. If the blood pressure in patients with hypertension and renal damage is reduced to the same absolute level as the normotensive patients, then more marked depression of renal function (GFR) may occur. This was seen in several instances in the present study and suggests that in the therapy of hypertension associated with renal damage, treatment should be aimed at post-drug blood pressures somewhat short of normotensive levels. If decreased excretory capacity follows blood pressure reduction with hexamethonium, the pressure should be allowed to increase to such a point that excretory function is not impaired in excess of that which is due to existing anatomical damage. This can best be evaluated on a long-term clinical basis by closely observing the level of blood urea nitrogen. However, the present studies were not designed to establish the long-term therapeutic efficacy of hexamethonium.

The cause of the decreased water and sodium excretion is not apparent. It is not due to an increase in the number of functioning nephrons available for sodium and water reabsorption since TmPAH was not altered by ganglionic blockade and blood pressure reduction. It cannot be explained solely on the basis of reduced glomerular filtration since in many instances it occurred independently of this function. In addition, one

hour after hexamethonium was administered glomerular filtration had returned to the control levels in most of the patients, but a reduction in the rate of water and sodium excretion persisted. It apparently was not due to the liberation of endogenous antidiuretic hormone since this hormone does not increase sodium reabsorption in man (11). Other investigators (12–14) have found that activity of the sympathetic nervous system alters tubular reabsorption of sodium and water. Renal denervation (13) in an unanesthetized dog did not affect GFR or electrolyte excretion, suggesting that the sympathetics had very little to do with this regulatory mechanism when the animal was not under stress. Sartorius and Burlington (14) found an increase in sodium and water excretion following denervation of the kidneys in anesthetized dogs, apparently due to the release of sympathetic neurogenic influences which are present in the anesthetized animal. A similar response was found by Moyer and his associates (9) after autonomic ganglionic blockade with hexamethonium in anesthetized dogs. Because of the possibility that anesthesia may previously have produced a sympathomimetic effect on the dogs' renal dynamics, which was released by ganglionic blockade with hexamethonium, these studies have been repeated on three unanesthetized dogs. The results using the same techniques as previously described are presented in Table V. In these unanesthetized dogs (Table V), GFR and renal plasma flow increased slightly as the blood pressure was reduced in each of the three animals thus studied. This was associated with an increase in sodium excretion. There was no essential difference between these observations and the previous ones using anesthetized animals suggesting that even in the unanesthetized animal, the autonomic nervous system may alter tubular reabsorption of water and electrolytes. These observations as well as the previous ones indicate that sympathetic neurogenic impulses to the kidney may cause an increase in tubular reabsorption of sodium and water in the dog. This is in contrast to the response of the patients observed in the current study, wherein ganglionic blockade (partial) enhanced sodium and water reabsorption rather than decreased it. It is possible that sodium and water excretion was depressed after hexamethonium was given because of sympathetic stimulation to the renal nerves, a result of the renal nerve ganglia not being blocked as completely as were the vasoconstrictor nerves elsewhere, thus causing a differential in vasoconstrictor activity within the sympathetic nervous system. In this instance, reflex vasoconstrictor impulses would emanate over the renal nerves as the blood pressure decreased, thus producing an initial sympathomimetic effect on the kidney. Vasoconstrictive impulses of this nature would also explain the initial reduction in glomerular filtration which occurred but which returned to normal despite a maintained reduction in blood pressure. If this were so, the difference in response between the present studies and the previous observations on dogs would merely indicate less complete blockade in the humans. However, it is quite possible that the difference in the type of response may be due to species differences.

Observations in the present study indicate very little, if any, inhibition of potassium excretion. It is of interest to note that potassium excretion did

TABLE V

Effect of hexamethonium on renal hemodynamics, electrolyte and water excretion of unanesthetized dogs

Dog No.	GFR		RPF		Na Excr.*		K Excr.*		UV†		BP	
	C	D	C	D	C	D	C	D	C	D	C	D
1	29	39	104	166	0.10	1.82	0.64	1.75	0.4	1.3	125	100
2	53	58	112	130	0.25	1.19	0.27	2.45	3.6	1.6	165	120
3	43	48	130	167	0.16	1.31	0.36	3.30	2.9	1.4	150	125
Mean	42	48	115	154	0.17	1.44	0.42	2.50	2.3	1.4	147	115

C—Control.
D—Average of 2 successive periods after hexamethonium.
BP—Mean blood pressure, mm. Hg.
* Milligrams per minute.
† UV, urine volume, ml. per minute.

not change as the urine volume decreased, indicating that potassium reabsorption did not follow the same pattern as sodium. It also indicates that changes in potassium excretion were not dependent on changes in urine volume in this instance. Otherwise, potassium excretion would have more closely paralleled the changes in urine volume.

SUMMARY AND CONCLUSIONS

1. Hexamethonium is an effective autonomic ganglionic blocking agent for reducing the blood pressure in both normotensive and hypertensive patients. The percentile reduction in systolic and diastolic blood pressures is about equal.

2. When the blood pressure is reduced with parenteral hexamethonium, there is an immediate reduction in glomerular filtration rate, sodium excretion and water excretion. However, after one hour, despite a maintained reduction in blood pressure, glomerular filtration rate returns to or approximates the control values. The antidiuretic effect persists but sodium excretion increases. At the point of maximum depression of blood pressure (D_2) there is not a consistent relationship between the reduction in glomerular filtration rate and the decreased excretion of water and sodium. Urine volume and sodium excretion are depressed proportionally until the urine volume is decreased to 20 to 30 per cent of the control values or less, and then the urine volume decreases more than sodium excretion. The initial effect on the kidney has the appearance of sympathetic renal nerve stimulation.

3. Kidneys showing marked impairment of renal function respond to blood pressure reduction with hexamethonium in essentially the same way as do normal ones, indicating that the nephrons are destroyed as anatomical units, and those that remain, function in a relatively normal fashion. However, this adjustment in patients with renal damage occurs more slowly than it does in patients with normal renal function.

4. The decrease in glomerular filtration following hexamethonium is not due to a decrease in the number of functioning nephrons since maximum tubular function (TmPAH) is not altered.

5. Moderate reduction in the blood pressure of patients with moderate to marked impairment of renal function is feasible. In most instances the functional nephrons that remain in damaged kidneys show a slower but a relatively normal hemodynamic readjustment to a lowered blood pressure.

6. After reduction in blood pressure with hexamethonium, norepinephrine is an effective vasopressor agent. The effect of norepinephrine on glomerular filtration under these circumstances is variable. If the reduction in blood pressure is excessive and glomerular filtration markedly depressed, raising the blood pressure with norepinephrine increases the filtration. If glomerular filtration is not depressed as a result of hexamethonium, increasing the blood pressure with norepinephrine does not affect or may slightly reduce glomerular filtration.

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DIETARY TREATMENT OF HYPERTENSION. III. THE EFFECT OF PROTEIN ON APPETITE AND WEIGHT

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In an earlier study (1) it was observed that patients rapidly lost weight following a change in diet from a conventional pattern to the rice-fruit diet of Kempner. Although the imbalance was but temporary, as shown by the approach to stable weight and nitrogen balance during the next four months, the weight remained at the lower level. During the period of rapid weight loss, the patients took a reduced number of calories as compared to their previous consumption. However, as the weight approached stability, the intake of calories rose to the neighborhood of the control values, despite the fact that the patients by this time were heartily bored with the menu. These observations and the finding that basal metabolic rates remained approximately constant, suggested that the daily expenditure of calories remained constant, but that the metabolic transition somehow required the oxidation of a certain bulk of tissue. It seemed of interest to plan further studies to isolate the dietary factor responsible for the transition, and to assess its importance in the therapy of hypertension.

In the subsequent study (2), which was directed mainly to the role of sodium, it was found that extreme changes in the ration of sodium had no important effect on the appetite or weight. Again in this study, the patients lost weight when given the basal diet despite the fact that all but one received a supplement of salt (10 gm. per day). The one patient who received no supplement exhibited a loss of weight which was indistinguishable from the others. It was surmised that the restriction of dietary protein to about 35 gm. per day, which appeared as an incidental restriction when dietary sodium was limited to 4 mEq. per day, was the factor that caused the reductions in appetite and weight.

In the present study this hypothesis has been given a systematic test. Essentially the same basal diet has been employed, with the addition of a salt-free milk product which permitted large variations in the intake of protein while the ration of sodium was held constant. The results support the hypothesis that a regulation of dietary protein may determine appetite and weight. These effects were clearly separable from the therapeutic action of limiting sodium, since the restriction of protein had no effect on the mean blood pressure at either a high or low intake of sodium, and an antecedent loss of weight did not condition the subsequent response to restriction of sodium.

PROCEDURE

Six patients with uncomplicated hypertension served as subjects for the present study (Table I). A careful history and a complete physical examination was recorded for each patient. Signs of cardiovascular disease, in addition to hypertension, were limited to enlargement of the heart and to grade I to II changes in the fundi. Patients with cardiac failure, history or neurological evidence of central nervous system disease, or with kidney disease, were not taken for the study. After the patients were admitted to the metabolic ward, the following diagnostic tests were done: urinalysis, urea clearance, intravenous pyelogram, complete blood counts, plasma protein concentration, serology (Kolmer and Mazzini tests), electrocardiogram, X-ray of chest and the benzodioxane test of Goldenberg and his associates (3). The tests confirmed the absence of complicating disease. During the course of the study several tests were repeated at intervals of one to four weeks: urinalysis, urea clearance, plasma protein concentration, electrocardiogram, and X-ray of chest; the results accorded with those on admission.

All of the food during the six months of observation was supplied in weighed portions from a diet kitchen, any surplus left unconsumed being reweighed and subtracted. With the aid of standard tables (4, 5, 6) the daily consumption of protein, carbohydrate, fat, total calories and sodium were computed. The basal diet was similar to the one used in the preceding study (2); it contained approximately 4 ± 1 mEq. of sodium and 35 ±

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[2] Fellow of the American Cancer Society as recommended by the Committee on Growth of the National Research Council.

TABLE I

Clinical data

Patient	R.I.H. No.	Age	Sex	Known duration of hypertension	Ratio Initial wt. • / Normal wt.
		years		*years*	
A	12596	36	F	2	0.88
B	12549	32	F	8	1.00
C	12575	48	M	21	0.86
D	12564	36	M	4	1.16
E	12566	45	M	4	1.20
F	12582	24	F	0.3	1.06

• "Normal weight" for each patient was calculated from normal values which had been derived from life insurance statistics (13).

5 gm. protein per day. The different categories of food supplied calories in the approximate proportions: protein 5 per cent, carbohydrate 70 per cent, fat 25 per cent. When an increase in the intake of sodium was desired, 10 gm. NaCl per day were given in gelatin capsules. The ration of protein was increased above the level of the basal diet by addition of a supplement;[3] it was reduced by substituting for the basal diet one of minimal protein content (8 gm. per day). Throughout the entire study, including the periods of extreme restriction of protein, an unlimited supply of carbohydrate and fat was offered to each patient; in this way the total number of calories taken each day remained under the control of the patient's appetite.

In the first period, which was of some 6 to 7 weeks duration, the intake of sodium was held at 175 mEq. per day by the addition of salt in gelatin capsules, and the intake of protein, around 1.5 gm. per kg. day. During the second period, again of 6 to 7 weeks duration, the intake of sodium was dropped to 4 mEq. per day without other changes in the regimen. In the third period, 5 to

[3] "Lesofac" (Wyeth, Inc.): 50 per cent milk proteins, 39 per cent carbohydrate, 1 per cent fat, 0.02 per cent sodium.

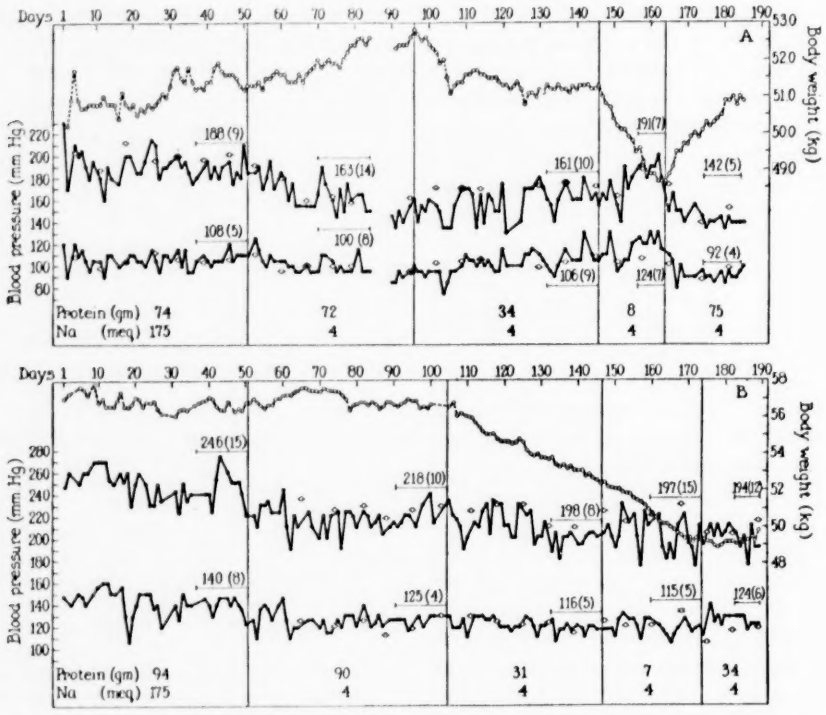

FIG. 1. DAILY VALUES OF MORNING WEIGHT AND BASAL BLOOD PRESSURES OF PATIENTS A AND B

The weight curve (upper line) is referred to the ordinate scale at the right; the two lower curves, giving systolic and diastolic pressures, refer to the scale at the left. The mean and standard deviation of blood pressures are given with horizontal lines that indicate the span of the averaging. Light circles amidst the curves of blood pressure indicate the values that were obtained under the non-basal conditions of service rounds.

TABLE II

Metabolic data

Blood pressure values are the means and standard deviations of basal pressures over periods of two weeks

Patient	Period	Average diet Na	Prot.	Cal.	Final weight	Blood pressure Systolic	Diastolic
	days	*mEq./day*	*gm./day*	*cal./day*	*kg.*	*mm. Hg*	*mm. Hg*
					50.7		
A	51	175	74.1	2,368	51.3	188 (9)	108 (5)
	45	4	71.9	2,332	52.8	163 (14)	100 (8)
	50	4	34.2	1,715	51.2	161 (10)	106 (9)
	18	4	7.9	1,244	48.7	191 (7)	124 (7)
	22	4	74.9	2,264	50.9	142 (5)	92 (4)
					57.0		
B	50	175	93.6	2,275	56.8	246 (15)	140 (8)
	49	4	90.2	2,147	56.7	218 (10)	125 (4)
	42	4	31.4	1,358	52.6	198 (8)	116 (5)
	27	4	7.2	908	49.3	197 (15)	115 (5)
	14	4	33.5	1,465	49.8	194 (12)	124 (6)
					55.2		
C	48	175	76.3	2,003	53.9	197 (8)	111 (4)
	45	4	75.2	2,056	52.1	160 (9)	101 (6)
	86	4	34.2	1,791	48.9	163 (9)	103 (7)
					79.9		
D	43	175	120.2	2,875	79.2	200 (8)	133 (6)
	44	4	116.3	2,501	76.2	154 (7)	95 (7)
	35	4	36.0	2,015	73.1	175 (14)	114 (10)
	55	4	120.0	2,924	74.4	187 (10)	130 (6)
					79.3		
E	50	175	120.4	3,012	78.7	186 (8)	131 (12)
	51	4	118.9	3,033	77.7	177 (12)	118 (8)
	74	4	36.5	2,102	69.9	166 (8)	110 (5)
	45	175	128.3	3,252	71.2	180 (13)	112 (9)
					55.5		
F	50	175	77.0	2,016	56.3	157 (3)	105 (4)
	42	4	76.6	2,106	55.6	143 (7)	94 (7)
	86	4	31.2	1,593	50.8	140 (6)	94 (3)

* Asterisk indicates a significant difference between consecutive values.

12 weeks in duration, the supplement of protein was discontinued, so that the patients subsisted on the basal diet alone. Two patients (A and B) thereafter were given brief trials of treatment with the diet of minimal protein content (0.15 gm. protein per kg. day), and then were returned to the basal diet.

As in the previous studies (1, 2), the patients were weighed to a precision of ± 100 gm. each morning after they had voided and before breakfast. The basal blood pressures were determined each morning after the patients had remained quietly in bed for at least one hour. As an additional measure of the blood pressures, readings were made by 3 to 5 physicians during the weekly service rounds. It had been expected that these would reflect the greater emotional stress of the occasion; on the contrary, the average values that were obtained during rounds were not significantly higher than the resting pressures that had been obtained earlier in the morning (Figure 1). An analysis of variance (7) showed that the difference between these two measures of pressure was no greater than would be expected from the variance between the values of resting blood pressure on consecu-

tive days. This result suggests that the average blood pressures during the moderate stimulation of ward activity were near the basal values.

ANALYSIS OF DATA

Appetite and weight

Changes in the appetite and weight of the patients were produced consistently by changes in the ration of protein. With decreases in protein from a high (1.4 to 1.6 gm. per kg. day) to an intermediate (0.5 to 0.7 gm. per kg. day) level or from intermediate to low (0.15 gm. per kg. day), a reduction in caloric intake soon followed; with increase in protein a greater number of calories were taken (Table II). The relation between these two variables is given by the regression equation, $C = (1116 \pm 97) + (14.6 \pm 1.2)$ P, where C and P are calories per day and gm. protein per day, respectively (Figure 2). The most interesting feature of this equation is the magnitude of the slope, 14.6 cal. per gm. protein. This is considerably greater than the caloric value of protein alone, 4.1 cal. per gm. protein; therefore a variation in the dietary protein was attended by a proportional variation in the quantity of non-protein constituents. The reason for such a correlated variation is not immediately evident, since each patient was allowed an unlimited intake of low protein foods, and thus remained in control of total caloric level. During the period of adaptation to a new level of dietary protein, therefore, the appetite as measured by this self-determined intake of calories was systematically influenced by the daily ration of protein.

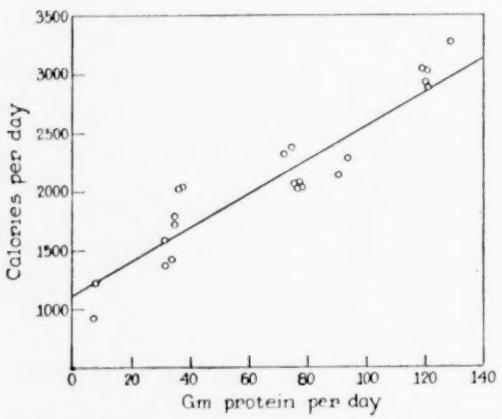

FIG. 2. RELATION BETWEEN THE SELF-DETERMINED INTAKE OF CALORIES AND THE PRESCRIBED RATION OF PROTEIN DURING THE PERIODS OF METABOLIC TRANSITION THAT FOLLOWED CHANGES IN THE RATION OF PROTEIN

The line corresponds to the regression equation, $C = (1{,}116 \pm 97) + (14.6 \pm 1.2)$ P, where C and P are calories per day and gm. protein per day, respectively.

It might be supposed that the increase of caloric intake with increase of dietary protein was merely an expression of the specific dynamic action of proteins. This is far from the case, however, since even the high value of 33 per cent for specific dynamic effect of added protein would imply an added output of heat of only 1.35 cal. per gm. protein, less than one-tenth the actual value. Indeed, the changes in body weight prove that the variations in quantity of dietary protein had more profound effects than simple changes in metabolic rate.

It may be seen in Table II that a reduction in dietary protein was followed invariably by a reduction in body weight, and an increase in protein by an increase in weight. These variations in weight were not due merely to changes in hydration; a gain or loss of subcutaneous fat was evident to inspection when the observations were extended over several weeks. Therefore, since a positive caloric balance was required for the weight gain during the periods of high protein feeding and negative balance must have prevailed during the restriction of protein, the feeding of protein must have stimulated a consumption of calories in excess of expenditure, and the restriction of protein must have depressed the consumption below expenditure. The negative caloric balances, shown at intakes of 0.5 to 0.7 gm. protein per kg. day, moreover, were not due to inability to attain nitrogen equilibrium at this level; in a previous study (1) it was shown that similar patients, given sufficient time, approached nitrogen balances at even lower levels of consumption.

BLOOD PRESSURE

It had been observed (2) that patients remained hypertensive during control periods in which the basal diet was supplemented with sodium chloride (10 gm. per day), and responded to omission of the salt with declines in blood pressure. Although these results illustrated the fact that restriction of

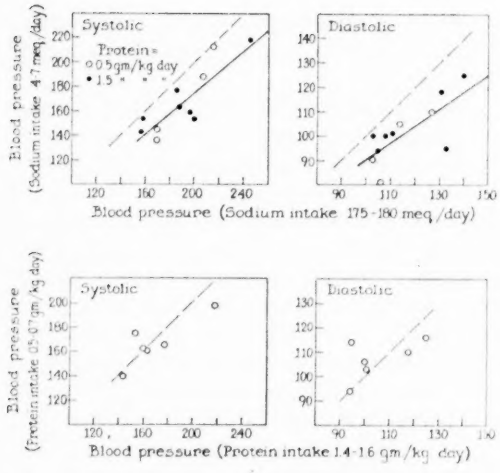

FIG. 3. CORRELATION OF MEAN BASAL BLOOD PRESSURES BEFORE AND AFTER CHANGE IN A SINGLE DIETARY FACTOR

The upper pair of graphs show the response to reduction of dietary sodium; the points shown by open circles refer to patients who experienced the reduction of sodium after a control period in which dietary protein was low, while the solid points refer to comparable patients who received supplementary protein. The dotted lines show the mean positions to be expected if the dietary change were without effect; the solid lines, showing linear regressions, are significantly different. The lower pair of graphs show that change in the level of dietary protein was without effect on the blood pressure of patients who received approximately 4 mEq. sodium per day.

sodium is necessary for a therapeutic effect, they did not answer the possibility that the considerable losses in weight and the associated metabolic changes during the control periods may have conditioned the response to a subsequent withdrawal of sodium.

In the present investigation the dietary protein was maintained at a high level (1.4 to 1.6 gm. per kg. day) during both the control period and the subsequent period of salt restriction. As a result of the addition of protein to the basal diet, the patients maintained steady weights, or actually gained. Despite the differing effects on body weight, the two regimens produced comparable responses to the restriction of sodium (Figure 3).

As a further test of the matter, the intakes of protein were varied while the intake of sodium remained low (4 mEq. per day). Reduction of dietary protein to an intermediate level (0.4 to 0.6 gm. per kg. day) was without consistent effect on the blood pressure (Figure 3), although it caused reductions of body weight (Table II). Extreme reduction of protein (0.15 gm. per kg. day) was followed by a rise of blood pressure in one patient and no change in the other (Figure 1).

Statistical analysis of these results confirmed the impression that the quantitative reductions of blood pressure were unrelated to the state of protein metabolism within the limits studied. The mean basal pressures after reduction of dietary sodium proved to be simply proportional to the mean pressures of the control period. Expressed as percentage reductions of the control values, the decrements of systolic and diastolic pressures had an identical value, 12 ± 7.4 per cent. The high coefficient of variation (60 per cent) is in notable contrast to the uniformity of treatment; presumably it reflects a variable capacity to respond.

DISCUSSION

Appetite and weight

These results show that appetite and body weight can be controlled under some conditions by changes in the amount of dietary protein. This effect is to be expected when any essential factor in the diet is reduced enough to limit metabolism; it is just such an action that defines the essential nature of a food. Usually in clinical nutrition, however, the limiting action of essential foods has been seen in the diseases that follow extreme restriction. In the present study a limiting action occurred on what must be considered an adequate diet, since diets even lower in protein suffice for nitrogen balance and maintenance of weight (1). If further proof of adequacy be needed, it can be pointed out that a large number, perhaps a majority, of the people in the world live, work and reproduce on diets that are at least as restricted.

It seems paradoxical that patients should lose weight when changed from one adequate diet to another. Two explanations are possible. The first and conventional one is that time is required for the organism to increase its efficiency, and during this period of adaptation, tissue is lost because of wasteful expenditure. An alternative is that loss of tissue is essential to the adaptation, being in fact a part of the metabolic adjustment.

The second explanation appears to us more probable. The organism maintains a steady state of composition despite the fact that it is composed of a vast number of substances in ceaseless flux, forming a set of interacting rate processes. Even a slight imbalance of rates would destroy the steady state. Certainly any change in the rate of one process would change the inventory of metabolites that are shared with other rate processes. Following such a change a new steady state might or might not be reached, depending on whether the changes in inventory bring the rates again into balance. In clinical nutrition a failure to reach a new steady state is illustrated by any severe deficiency which causes progressive deterioration and death. Milder limitations, such as were imposed in the present study, lead to a new steady state, but it should be emphasized, only after the necessary changes have been made in the inventory.

This explanation assumes that the dietary supply of an essential food may determine rates, even when the quantity of food is varied in a range above the minimum for a steady state. To some extent, at least, this must be true for all foods that are excreted in an altered form. In particular for protein, work with rats has shown that the quantity of protein in the diet has a marked effect on the quantity and metabolic activity of proteins in the liver, kidney and plasma (8).

The effect of the present diet on appetite may be given two different kinds of explanation. The first is that the diet is monotonous or uninteresting, and so the patients just eat less. Although this seems to be a reasonable statement, it really is not an explanation since it merely restates the result. The second is that the appetite responds to the state of metabolic inventory in such a way that the organism is motivated to eat when the inventory has been depleted and to reject food when items have accumulated. If we assume this, we can trace a sequence of cause and effect in the response to lowered dietary protein. The first effect of lowered dietary supply is a reduction in metabolic activity of proteins and derived metabolites. Because of the imbalance of rates, some of the other metabolites come to be in relative surplus. The appetite, therefore, is depressed and the inventory of the other metabolites is allowed to shrink until a new steady state is reached, a matter of several months in the case of protein restriction (1). Then the appetite returns to a balance with expenditure.

A similar explanation might apply to the high fat and protein reducing diet that was introduced by Pennington (9). In this case the carbohydrate seems to be restricted, and calories in the form of fat, available without limit. Reducing diets of this type, working by an effect on appetite, seem to be more strategically planned than the conventional diets which merely deny calories. Metabolic imbalances probably are inescapable during ing the transitional period of weight loss. Conventional diets with an arbitrary fixation of the whole pattern of intake would seem to offer little scope for compensation. Diets of the sort that control through a limiting factor leave many degrees of freedom to the appetite, and it is reasonable to suppose that the freedom is wisely used. At any rate, it is reported (10) that the usual weakness and hunger of weight reduction are absent on the high fat and protein diet. The present study gave similar results; none of the patients losing weight on the low protein diets admitted hunger or weakness. This, or a similar diet, might have some value in the treatment of obesity.

Blood pressure

The data support a conclusion of earlier reviewers (11) that the level of dietary protein does not influence the initiation or the maintenance of clinical hypertension. In this respect the results diverge from those reported by Handler and Bernheim in a study of experimental renal hypertension (12). Rats, made hypertensive by constriction of one kidney, became normotensive on rations that provided a normal intake of sodium, but which were restricted either in the quantity of protein or total calories; restriction of sodium, on the other hand, had but little effect on the blood pressure of rats on high protein rations. The discrepancy between the clinical and the experimental results might be due either to the difference in species or to a difference in etiology of the hypertensions.

SUMMARY

The reduction of blood pressure following restriction of dietary sodium was found to be in-

dependent of tenfold variations in the ration of dietary protein, and unaffected by prior or subsequent reduction of body weight. The data indicate that restriction of dietary sodium may be expected to reduce the systolic and diastolic blood pressures by 12 ± 7.4 per cent.

The ration of protein, under the conditions of this study, was seen to determine the appetite and the body weight of each patient. It might seem odd that patients lost weight when changed from one adequate diet to another, but the result can be explained on the hypothesis that the reduction of body mass is an adaptation to the reduction of dietary protein. The lack of hunger or weakness during the loss of weight indicated that the diet might be of some use in the treatment of obesity.

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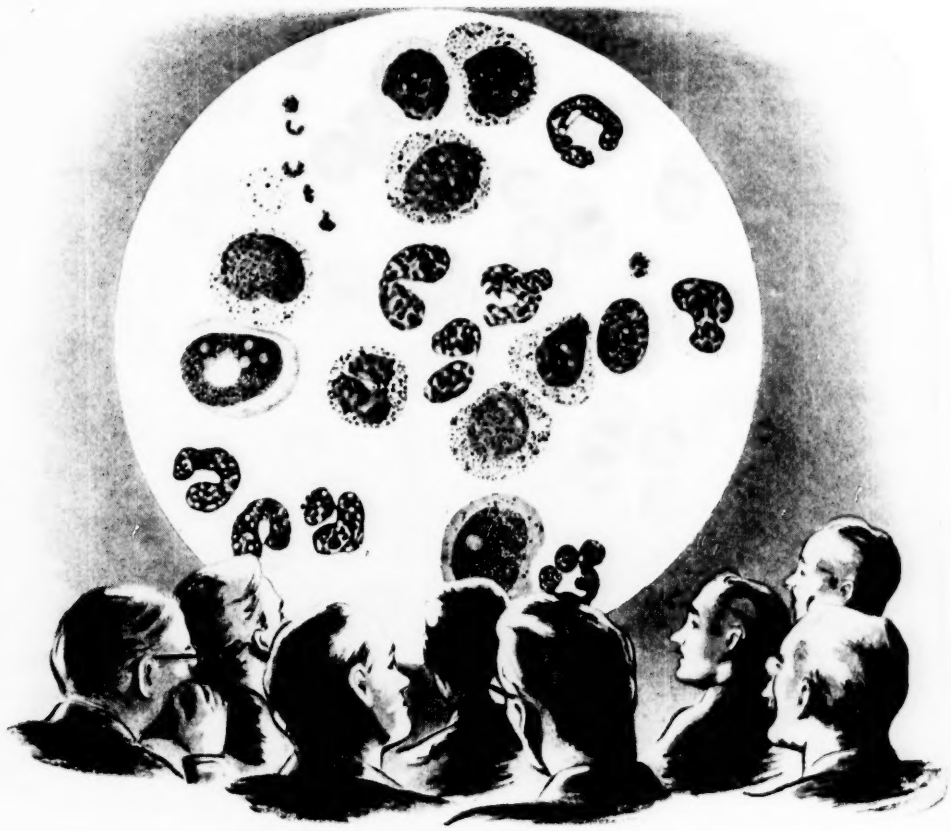

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